

Right and wrong ways of expressing concern

WE publish a letter from Professor F. Janouch on page 181 which together with the appeal from Professor B. Peleska to the World Federation of Scientific Workers (WFSW) (*Nature*, July 12) makes for most disturbing reading.

For a long time there has been talk of the miseries of some members of the academic profession in Czechoslovakia whose political views did not accord with those of the government. Much of this talk, however, has been coupled with a fear that to name names in the West would simply lead to greater personal distress in the East. And some of the talk has been simply of a subjective nature from visitors— that Prague has become 'the most miserable of capitals' and so on. Fear of naming names is not ill-founded; Professor Peleska has already, it is said, been visited by the police in connection with the letter sent to the WFSW and reprinted with his permission in *Nature*.

Czechoslovakia then, according to many with whom we have spoken, is at present about as bad a place as any to be an academic devoted to the spirit of liberal enquiry and freedom from repression. What can academics do in a public sense about preventing a breakdown in the standards which they regard as proper for the pursuit of learning? There are, we believe, no essential differences in approach between concern for Czechoslovakia and concern for, say, Chile.

It is easier, of course, to spell out what they shouldn't do.

- They shouldn't assume that an intellectually distinguished body is capable of leading the way diplomatically. The Royal Society in Britain, for instance, has been under much criticism in recent years for its failure to come out publicly on such issues as Soviet treatment of certain scientists. The standard defence is that much good work is done behind the scenes in person-to-person meetings with opposite numbers. This may well be true, but surely the real answer is that a society founded on intellectual distinction should not be asked to do a job requiring a high degree of political acumen.

- They shouldn't expect a politically committed body to deliver the goods. The WFSW has done some excellent bridge-building in the past and may appear, through its clear links with Eastern Europe, to be just the right vehicle for transmitting criticism. But the links with the East have been at the expense of strength in the West and particularly in the United States where an enormous number of scientific workers are and yet where few have even heard of the federation. It is sad that Professor Burhop, president of the federation, should have chosen to respond to the appearance of the Peleska letter by counter-examples of western victimisation, but it shows the weakness of any organisation which as the Roses put

it in *Science and Society* is a victim of cold-war politics and East-West polarisation.

- They shouldn't confine their concern to their own specialisation. An academic community divided into science, sociology and the arts or whatever is weakened, may be to the point of ineffectualness. The world of learning must be indivisible on such matters.

But what positive steps are there to recommend if so often academics have used the wrong channels for their protest? Two seem to be under-explored and worthy of more attention—the approach to politicians and the use of the press.

The elected politician with either his access to or his desire to pose problems for, a government has been underestimated as a means of applying pressure in shaping foreign policy. And the ability of foreign policy to include specific references to the problems of oppressed groups has been inadequately used by academics—although a start has recently been made in the inclusion of concern for Soviet Jews in US foreign policy. If a hundred professors in any one country took individual initiatives to harangue their representatives on a matter so obviously not involving self-interest, could that country's foreign policy remain unaltered?

If approaches to politicians might help on a national front, approaches to the journals could rapidly move information around the world in order to create the necessary international climate. Who can doubt that wide publicity of the preposterous behaviour of the Soviet authorities in excising names that displease them from published papers serves a valuable purpose. And the scientist in particular, wedded to the importance of documentary evidence is at once more able to appreciate hard fact and more likely to be able to supply it. It is all-but-impossible for an editor to resist a well-documented story.

A hundred years ago



THE will of the late Girolamo Ponti, of Milan, which has just been published in the *London Gazette* by order of Lord Derby, is likely to give rise to some trouble before it can be carried into effect. The testator has bequeathed a considerable portion of his property to the "Academies of Science of London, Paris, and Vienna," to be divided among them in equal proportions, for the purpose in each case of founding, with the proceeds resulting from investment, two competitions yearly on the subjects of Mechanics, Agriculture, Physics and Chemistry, Travels by Sea and Land, and Literature. The committees to be appointed by the societies are instructed to give preference to those competitors who will have advanced any of the subjects mentioned by original discovery. The relatives of Signor Ponti are to dispute the will, and those London societies that think they have claims upon the legacy are urged to bring them forward at once. There can be no doubt which societies are meant in the case of Paris and Vienna; and at first sight there appears to be little doubt as to what body the title of "Academy of Science of London" would most appropriately apply.

From *Nature*, 10, 427, Sept. 24, 1874.



It has been claimed on a number of occasions during the past few years that it is possible to bring about substantial improvements in the health and well being of the poor people of the world by the application of some relatively cheap and simple technology. Such a possibility seems theoretically doubtful and we feel that it is most important that attention should not be distracted from the real problems of social and economic developments by exaggerated claims for the impact of some particular technique. It is for this reason that Aaron Altschul's restatement of the apparent benefits of fortification of foods with amino acids¹ needs a critical appraisal.

We might begin by pointing out the significant fact that although the advantages of adding amino acids to human foods have been vigorously promoted for at least 20 years, and the technology for doing so has been available for as long, there is to date not a single example of a controlled field trial on a population basis which has shown any measurable benefit to man. We believe there are both nutritional and logistic reasons for this.

The nutritional basis for amino acid fortification rests upon the proposition that a specific deficiency of protein is a common cause of ill health. Such a statement requires definition. It is important to distinguish between the physiological needs for protein as a nutrient and the desire for foods such as meat, milk, eggs and fish. It has been a consistent feature of the arguments of those who advocate protein or amino acid fortification as a cure for malnutrition that they confuse the problem of meeting physiological needs for protein with the problem of meeting demands for aesthetically satisfying diets. Perhaps it is not surprising that many people can see no way in which the demands of the rich for meat could (or even should) be moderated in the light of the needs of the poor for bread. It is surely hypocrisy to pretend on the one hand that these demands

Strategy for a hungry world

Dr D. S. Miller of Queen Elizabeth College, and Dr P. R. Payne of the London School of Hygiene and Tropical Medicine take issue with a recent article in Nature on the value of amino acid supplementation, especially in developing countries.

and needs are both expressions of the same protein problem, and on the other to suggest that the needs of the poor can be satisfied by fortification of staple foods with a tasteless, odourless powder which will help neither to satisfy hunger or incidentally to improve the nutritive value of the diet.

The second problem of definition is to distinguish between diets which are 'limited' by a particular amino acid and those which are 'deficient'. For example the traditional English breakfast of porridge, bacon and eggs, toast and coffee contains a protein mixture that if fed under carefully controlled conditions could be shown to be limited by methionine. But this is neither evidence that the UK population is at risk from methionine or protein deficiency, nor does it justify the fortification of breakfast foods with amino acids. A diet may only be considered deficient in an amino acid if it contains less than the requirements of the consumer.

Dr Altschul correctly points out that standards of protein requirements are now "lower than before". What he does not point out is that one takes the latest FAO/WHO standards², it can be shown that the required percentage of calories from protein in mixed diets as eaten by man is only 8%. This assumes

that the efficiency of protein utilisation of human diets eaten in poor countries is about 65%. In fact, the calculated efficiency derived from the methods given by the FAO/WHO report is higher than this at about 90% which would lower the required percentage of dietary protein even more. Thus the cited projection that the protein content of the diets eaten in South Asia will fall from 10.2% to 9.8% by 1980 does not indicate impending disaster. Of course there are those who argue that the poorest people are eating bread or rice alone. These cereals will have an efficiency of utilisation about 50%, and hence the required protein content of the diet would rise to 10%, still covered by cereals alone. Another argument is that cereals do not contain enough protein for the vulnerable groups in the population such as pre-school children. But the latest requirements indicate that they also require only 10% cereal protein as a percentage of the calories. Thus simple protein deficiency cannot occur in areas where cereals are the cheapest food, and amino acid supplementation would therefore be a wasted effort. Simple protein deficiency can only occur where the staple food contains less than 10% protein such as cassava, sago, and plantain. Since these foods contain virtually no protein, supplementation with a few amino acids cannot increase their useful protein content by changing protein quality.

This is not to say that there is no such thing as protein-calorie malnutrition. This syndrome arises chiefly because children do not eat enough food. It is complicated by disease, especially diarrhoea, respiratory infections and measles, but the prime cause is insufficient food. We were interested to read in the same issue of *Nature*, the account by Ethiopian relief workers and their critical assessment of protein-rich foods. Indeed your editorial correctly praised this group for their design of an energy-rich food for use in such situations. It is clearly non-



sense to supply developing countries with single nutrients, such as amino acids or vitamins, when some people are unable to buy and consume enough of the traditional diet which has sustained the population for centuries.

Turning now to the logistic problems of amino acid fortification, we would suggest the following conditions before any project should be implemented.

- That the limiting amino acid in the diet as distinct from the staple food, must be known. The extent of the limitation must also be known. In practice, cereal-based diets as eaten are found not to be limited by lysine. Miller and Donoso³ have shown that the limiting amino acid of most diets, including cereal diets, is methionine. Also that the improvement in the effective protein content when methionine is added is trivial in comparison with the data cited by Dr Altschul concerning the effect of the addition

of lysine to flour alone.

- That the supplemented food is consumed by the vulnerable groups in the population. This is normally only practicable where the staple foods are centrally processed and almost impossible where the major proportion of the population are subsistence farmers.

- That the project is the cheapest method of improving the diet as a whole. Since the chief cause of malnutrition is poverty, amino acid supplementation should be regarded by governments as an alternative to raising the standard of living. Thus to compare a scheme for adding lysine to wheat with one for improving milk production from marginal grassland, purely on the basis of the yield of protein is misleading. Improving agriculture provides employment, can be achieved within the country, and produces a product which is both aesthetically satisfying and acceptable. Finally

we must point out that Dr Altschul does not fully quote one of his references⁴, which indicates that the cost of fortification of all cereals in India would be no less than 16% of the Central Government Budget, or approximately five times the money spent on health.

We conclude that amino-acid fortification of staple foods should be rejected on nutritional and logistic grounds. The best solution to the problem of malnutrition is to raise the standard of living and to improve traditional agriculture. Aid to the subsistence farmers is the best way to achieve both.

¹ Altschul, A. M., *Nature* **248**, 643 (1974).

² FAO/WHO Technical Report No. 522 (1973).

³ Miller, D. S., and Donoso, G., *J. Sci. Fd. Agric.*, **14**, 345 (1963).

⁴ Schertz, L. P., *Nutr. Rev.*, **31**, 293 (1973).

international news

THE World Health Organisation (WHO) has been carrying out allegedly secret research projects in India, financed by agencies of the U.S. government. It has recently come to light that the U.S. Army has been an interested recipient of the research results from these projects.

The WHO experiments are being conducted (some have been completed) at the Genetic Control of Mosquito Unit (GCMU) in New Delhi, Bombay Natural History Society (BNHS) and the malaria eradication station in Jodhpur (Rajasthan state). The projects in question relate to the genetic control of mosquitoes, bird migration and the like—seemingly scientific investigations, till one takes a closer look at the related details. But Indian experts feel that the experiments could also provide data on the availability, behaviour and peculiarities of disease carriers in India—information that could be of value in biological or germ warfare.

Until recently the GCMU had been collecting data on the ecology, behaviour and dispersal patterns of mosquitoes in South Delhi and in Faridabad, a sprawling industrial complex near the capital. Some scientists have expressed fear that, using these data, an enemy could conceivably employ mosquitoes for the effective transmission of germs and viruses. Also, the GCMU conducts most of its experiments in and around Delhi—an area known not to be endemic for malaria or filariasis—instead of in the really

Doubts over US in India

from Narender K. Sehgal

endemic areas around the country.

The GCMU proposes to launch a big experiment early next year in Sonapat (again near Delhi) aimed at genetic control of *aedes aegypti*—in spite of the fact that the GCMU's first priorities ought to be malaria and filariasis carriers. Malaria is back in India and filariasis is endemic in an area occupied by a fourth of the Indian population. Dengue, unknown in India before July 1963 when it was detected for the first time in Calcutta, appears sporadically. Yellow fever is non-existent in India, not a single case having been reported in ages. This, it is argued, may be because of some 'balancing virus' that ensures protection against this disease. But the GCMU's genetic experiments could reduce or destroy this natural 'balance' and render the country vulnerable to yellow fever.

Indian scientists taking part in the GCMU's projects point out that their project leaders are, and have been in the past, U.S. government scientists. Two years ago, the GCMU attracted public attention when it was found to have been polluting village wells—

apparently in the course of scientific experiments—with chemicals suspected to be cancer-causing and prohibited in the USA. Since then secrecy around GCMU projects has been reportedly tightened.

Another controversy that has been raging among the scientific community here has to do with the role of Indian scientists and research organisations in programmes sponsored jointly by the Migratory Animal Pathological Survey (MAPS) of the U.S. Armed Forces Institute of Pathology and WHO. Reacting to criticism in the Indian Parliament the president of the Bombay Natural History Society, in a letter to a New Delhi daily, stoutly defended his society's collaboration with the MAPS on bird migration projects; he denied allegations of secrecy, ongoing collaboration arrangements with the MAPS and employment of U.S. personnel in bird migration studies.

Following this a scientist of the Jawaharlal Nehru University in New Delhi, in a letter to the same daily, pointed out a few things the BNHS president forgot to mention in his letter. Namely that (i) the blood samples of the birds caught by the BNHS were not analysed in India but were sent for the purpose to the Smithsonian Institute and the MAPS in the USA; (ii) two American scientists belonging to the Smithsonian were associated with the BNHS-MAPS programmes in India; (iii) the services of the BNHS and VRC (Virus Research

Centre of Poona) were made available to the U.S. Army agencies at nominal cost; (iv) the knowledge gained by the Indian scientists at BNHS and VRC was only partial, and probably the least significant if it did not include the details of the blood sample analyses and the overall data on bird movements and induced infections; and that (v) only a coordinating agency outside this country, which obtained comprehensive data from other units, could assess the military uses or significance of such experiments.

There is no doubt that the WHO projects in India are carried out with the approval of the government of India through its ministry of health, but when the matter of involvement of the U.S. Army in some projects was raised in the Rajya Sabha (upper house of the Parliament) the government merely assured agitated members that it would see to it that national interests were not endangered by these projects. This routine assurance to Parliament members has not impressed many in the scientific community, and demands are being made for a thorough probe into the whole situation. □

Science policy in the Netherlands

from Arie de Kool, Rotterdam

DISCUSSIONS on science policy are running so high in the Netherlands that even scientists are beginning to take an interest. The radical Bond van wetenschappelijke arbeiders (Union of Scientific Labourers) has been publishing reports on science policy ever since it was established about six years ago, and recently they have begun to co-operate closely with the Pugwash-based Verbond van wetenschappelijke onderzoekers (Union of Researchers). Together, the two unions have brought out a joint proposal for a democratic structure for science policy.

Their proposed structure is supposed to keep technocracy out, and guarantee a maximum amount of freedom and power over his own work for the research worker while at the same time safeguarding scientific standards. It is based on university organisation where one finds at the lowest level a section (for example, the solid state physics section or the French section), governed by a council on which scientific staff, technical assistants and students are represented (but in such a way that students can never obtain a majority). Several sections form a faculty, with its own council.

According to the new proposal the sections would not only join within the university, but all sections for, say, solid state physics, would elect a

"working community" at a national level. This working community would advise upward on the urgency of different programmes, and also receive money to pass out to the working groups in the universities. In fact working communities are now functioning rather well on the average, doing exactly this. There would be two differences; they would be elected by the scientists involved (and not appointed by the Dutch version of the National Science Foundation, ZWO, or one of its subsidiaries), and they would be responsible for about one third of the money going to a specific discipline.

Over and above the councils, we are to find a national council for higher education and scientific research. This is an almost purely political body, to advise government and parliament on matters contained in the name, and at the same time an executive body, handling all the money. One third of the research money would go to the universities to be spent at will (as long as it is for research; the democratic structure of the university is supposed to guarantee that this money is well spent). About one third goes to the universities, but would have to be spent in the faculties more or less proportionally to the number of students. And one third would go to the discipline councils to hand further down.

The idea behind this being that one needs a certain degree of centralisation if one wants any science policy at all. On the other hand, too much of it tends to kill the initiative, and close off new roads and new research, while there may be a strong tendency to overemphasise certain fashionable fields. In this structure, one would see about one third handled centrally, about one third at the university level, and about one third (the per-student amount) "at the basis", in the working group itself. At any level the researcher is supposed to have a certain degree of influence through his representatives, but of course, this private influence diminishes as one goes up higher in the structure.

Industry has been almost left out. The report says that since none of the proposed institutions could be expected to effectively exert power over industrial science, it would be wrong to have an industrial influence in the different councils. However, a better co-ordination of industrial and university research in socially relevant fields, is seen as worthwhile. In the long run government might even want to stimulate industrial research in those fields, and want to put a brake to other fields. Therefore industry should be asked to report on its scientific and technical activities, for instance, to the national council. □

Trouble in the air

by Colin Norman, Washington

FOR the past couple of years a number of industrial groups, taking their cue from the automobile industry, have been waging a pitched battle against some of the Federal government's plans for cleaning up air pollution. Their prime objective has been to persuade Congress to emasculate some key provisions of the Clean Air Act—the foundation of the government's air pollution strategy—which they consider unduly stringent, scientifically invalid and, of course, inordinately expensive.

But the rug was pulled from under industry's feet last week when the National Academy of Sciences unveiled a massive study of the Clean Air Act and its scientific foundation. Although the committee which conducted the study posed more questions than it answered, and hedged all its conclusions with a string of qualifications, it said that there is "no substantial basis" for weakening key provisions in the act, and that the costs of cleaning up automobile emissions are at least commensurate with the benefits.

A few Congressional staff members centrally involved in the debate over air pollution standards all said in response to inquiries last week that the Academy's study will probably provide Congress with sufficient ammunition to ward off challenges to the Clean Air Act which are certain to arise next year—the act is due for review and possible amendment in 1975.

The Clean Air Act sets maximum levels of various pollutants which are allowed in the atmosphere, the chief intent of these so-called air quality standards being to protect public health. In addition, the act specifies maximum levels of hydrocarbons and oxides of nitrogen which can be discharged by new automobiles. The air quality standards have, however, been attacked for being stricter than necessary to protect public health, and the automobile emission standards have come under fire for being too costly to meet.

But the Academy's study, which was conducted under contract to the Senate Public Works Committee, said that the standards have, in general, been supported by the evidence which has accumulated in the three years since they were set. Moreover, "the safety factors provided by the air standards are much smaller than is usual in regulating other environmental pollutants such as radioactivity", the study concluded.

In fact, using calculations which are admittedly uncertain but which are derived from a variety of sources, the Academy committee reckons that auto-

EVER since Gerald Ford was elevated to the Presidency early last month, there has been a good deal of speculation among members of the scientific establishment on whether or not he will resurrect some of the White House science policy machinery which his predecessor abolished. With galloping inflation and Presidential pardons keeping him occupied, Ford has, of course, shown no indication of rushing to deal with the matter, but there are a few suggestions that perhaps he might attend to it at some time.

First, he met earlier this year with a number of scientific society presidents and was reported to be receptive to the need for a scientific presence to be reestablished in the White House. Second, he was personally briefed on the recent report of the National Academy of Sciences which urged him to bring the science policy machinery back in from the National Science Foundation, which is where Nixon dispatched it. He was said to be a willing listener. And third, his nomination of Nelson Rockefeller as Vice President brings to the White House a man who is well known for seeking out science advice, albeit mostly from Edward Teller, the so-called father of the United States H-bomb.

But perhaps the most decisive move could come from Congress. Last month, the Senate Commerce Committee reported out a bill which would set up a Council of Science and Technology Advisers at the President's elbow. The bill has also been referred to the Senate Committee on Aeronautical and Space Sciences, which will probably approve it this week. There is thus a chance that the idea will get the blessing of the entire Senate before it adjourns in mid-October, in which case the whole matter will be brought rather abruptly to Ford's attention.

So far, however, nobody has been

able to demonstrate convincingly that the present arrangement is not working properly, or that a White House science policy office would work any better under Ford than it did under Nixon.

● OFFICIALS at the National Science Foundation are now trying to decide how to apportion a \$20 million budget cut which was recently imposed by Congress, but they have little choice except to take virtually all of it out of basic research.

Congress, demonstrating once again that its rhetoric in support of science and technology doesn't count for much when it actually comes to spending money, cut \$20.05 million from the

Washington seen

by Colin Norman

budget request for NSF, but also directed the foundation to spend some \$6.8 million more than it wanted on a few politically sensitive education programmes. The upshot is that \$26.85 million must be shaved from other NSF activities.

Congress itself dictated that at least \$5.5 million must be taken from the programme of Research Applied to National Needs (RANN), and that another \$1.4 million should come from a research management programme, but it left NSF officials free to decide where to cut the other \$20 million.

According to NSF budgetary officers, however, the only area that can be cut is the foundation's support of basic university science, and they reckon that as much as \$18 million could come from there.

● IN LANGUAGE uncharacteristically blunt for that decorous institution, the National Academy of Sciences has

criticised the Environmental Protection Agency's management of its research and development activities, and has suggested that EPA should scrap its present planning and management procedures and devise some new ones.

The criticisms were contained in a report drawn up by an ad-hoc Academy committee under the chairmanship of Dr Robert W. Berliner, former Deputy Director of the National Institutes of Health.

The committee found that planning of EPA's research and development programmes is separated from responsibility for their execution, which has led to "severe resentment" among the agency's scientists. Research priorities do not necessarily reflect the needs of EPA's regulatory officers, the planning system is excessively complex and detailed, there is no long term research programme designed to meet environmental needs, and "relationships between the headquarters and field (staff) are strained at best".

The committee also noted that the fact that EPA's research and development budget has been held constant for the past few years doesn't help matters.

To help remedy the situation, the committee suggests that the present arrangements should be scrapped, that the directors of EPA's laboratories should be given more of a say in the planning process, and that the headquarters staff should be trimmed by decentralising some of the control.

The report is the first to emerge from a \$5 million contract between EPA and the Academy under which the Academy will conduct a comprehensive study of EPA's activities. The study was mandated by Congress at the urging of Mississippi Congressman Jamie Whitten, a strong supporter of DDT and other pesticides, who believes that EPA is out to destroy American agriculture.

mobile pollution could now account for as many as 4,000 deaths and four million days of illness among Americans each year. That represents about one-eighth of the deaths from bronchitis, emphysema and asthma, and one-tenth of the days lost from work each year from respiratory illness.

The committee told Congress that it sees no reason to alter any of the air quality or automobile emission standards, although it does suggest that the standard relating to emission of oxides of nitrogen by automobiles "may be somewhat more stringent than is needed". But the automobile companies will find it difficult even to use that suggestion as ammunition against the Clean Air Act, since the Academy com-

mittee says in the next breath that since little is known about interactions between oxides of nitrogen and hydrocarbons in the atmosphere, it would be unwise to relax the standard at this time.

Finally, an amusing footnote to the debate over automobile pollution was provided last week by General Motors. When the exhaust emission standards were first set, the entire automobile industry rose up in arms because the timetable for meeting the standards was so short that they would have to invest huge sums of money in exhaust catalysts—no other technology would have been available in time.

Motor manufacturers argued vehemently that catalysts would be unre-

liable, that they would decrease engine performance, and that they would greatly increase gasoline consumption. But Congress refused to scrap the timetable and many of the 1975 model cars which still come onto the market this month will be equipped with catalysts. General Motors has consequently changed its tune.

According to a series of full-page newspaper advertisements which the company took out last week: "General Motors believes it has an answer to the automotive air pollution problem . . . and the catalytic converter has enabled GM engineers to improve performance and to increase miles per gallon." It's amazing what a little government pressure can do. □

Vancouver boycott supports Plyushch

from Vera Rich

THE right of a scientist to travel abroad for professional purposes is one of the most vexed questions confronting campaigners for human rights in the USSR. Recently the greatest attention has been focused on those Jewish scientists who wish to leave the Soviet Union permanently and settle in Israel—a desire usually denied either on the grounds that the scientist concerned has allegedly had access to classified information, or else that he represents an asset to the State in terms of education and experience. (The fact that scientists who apply for a visa to Israel tend to be fairly rapidly dismissed from their posts does not entirely vitiate the latter argument—the authorities may not wish to make use of the services of these valuable personnel, but they do not wish their services made available elsewhere).

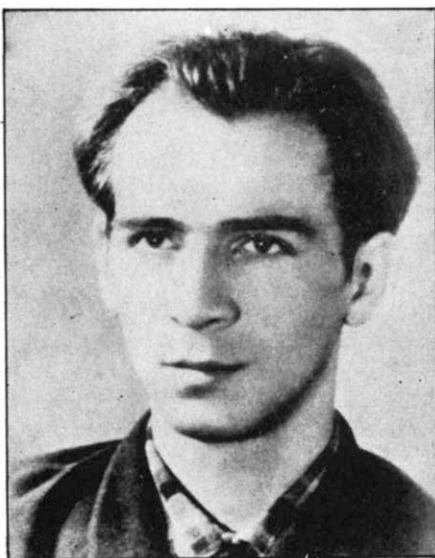
The case of scientists who wish for a temporary visa is far less defensible. These scientists simply wish to travel abroad to a conference, deliver their paper, and return home. There is no question of settling abroad; they wish merely to make a temporary visit. Yet all too frequently, invited scientists fail to arrive at conferences, and their places are filled by uninvited representatives of the Soviet Union, who have little or no reputation in the field concerned, and who insist on delivering papers which often have only a very peripheral connection with the subject of the conference.

This substitution of delegates can, however, possibly be turned to good account by those who wish to press for academic freedom for their Soviet colleagues. At the recent International Congress of Mathematicians in Vancouver, a number of participants decided to boycott the papers of such uninvited delegates as a protest against the persecution of a number of Soviet mathematicians, notably that of Leonid Plyushch, whose fate was also the subject of an appeal to the Congress by Dr Andrei Sakharov, the founder of the illegal "human rights" movement in the Soviet Union.

Plyushch, a mathematician and cybernetician, was formerly employed at the Cybernetics Institute of the Academy of Sciences of the Ukrainian SSR, specialising in the computer simulation of biological and biochemical processes. In 1968, he was dismissed from his post as the result of a letter which he wrote to the newspaper *Komsomolskaya Pravda*, protesting against the illegality of the trial of the writers Ginsberg and Galanskov. In 1969, he became a founder-member of the illegal "Initiative Group for the Defence of Human

Rights", founded by Dr Andrei Sakharov. In 1972, after almost four years without work, and a number of interrogations and harassments, Plyushch was arrested on January 17, 1972, under Article 62 of the Criminal Code of the Ukrainian SSR for 'anti-Soviet activities'. His arrest formed part of a general campaign against the *samizdat* journals *Chronicle of Current Events* and the *Ukrainian Herald*. In January 1973, he was sentenced *in absentia* to indefinite confinement in a special mental institution, suffering, it would appear, from that notable discovery of Drs Lunts, Morozov, *et al.*: "schizophrenia leading to ideas of reform making."

Since then he has been confined in the Dnepropetrovsk "special" psychiatric hospital, and an appeal dated



Plyushch: "at the border of death"

February 12, 1974, and signed by six leading intellectuals including Sakharov, which reached the West via the *Samizdat* network, speaks of the "appalling conditions of humiliation, persecution and physical suffering" in which he is held. Intensive "treatment" with haloperidol has caused a "sharp deterioration in his health, extreme exhaustion and continuous shivering, weakness, swellings, spasms, and loss of appetite." By that time he could no longer read, write letters or take advantage of the hour's exercise permitted to confinees. All requests by his wife for information on her husband's state of health, and treatment were refused.

Shortly after this appeal was drafted, an International Committee of Mathematicians for the Defence of Shikhanovich and Plyushch was set up in Paris. (Dr Yuri Shikhanovich is a Moscow logician who was being held on charges similar to those against Plyushch.) After a number of requests to the Soviet embassy in Paris for news of the two mathematicians, the Committee was in-

formed that the diplomats were "inadequately informed" in these matters, but that the Soviet Union never used confinement in a mental hospital as a punitive measure, and that the "special" hospitals such as that in which Plyushch is confined were established so that scientists could receive especially good treatment and care. Later requests produced the 'information' that Plyushch was dismissed for negligence in his work and for having lost departmental documents. After which he made no attempt to find further work, but engaged in writing and circulating anti-Soviet material. During the judicial enquiry into these activities he was found to be suffering from schizophrenia and was still (March 25, 1974) in need of medical treatment. A reexamination of Shikhanovich "in order to consider the possibility of terminating his course of treatment" was scheduled for March, and in June, he was in fact released, apparently in response to the Committee's pressures.

But Plyushch remains in confinement, perhaps on account of his Ukrainian nationality. Ukrainian dissidents, with their stress on equal rights for minority nationalities (and in particular for their own 50-million-strong 'minority') are always viewed considerably more severely by the Moscow authorities, lest nationalism should lead to a revival of Ukrainian separatism. (The Ukrainian SSR, like the other Union republics, does, of course, possess on paper the right to secede from the Soviet Union.)

Whatever the reason for his continued confinement, the conditions in which he is held according to the appeal of last February, have brought him "to the border of death", and even if he survives there is a real danger of irreversible psychological and intellectual damage.

It is not surprising, therefore, that appeals should have been made to the Vancouver Congress, both from the Paris Committee and directly from Sakharov, to "pass a resolution in Plyushch's defence, and to take all possible measures to save him." What is new is the suggested means of protest and the boycotting of uninvited papers. A great deal may be said in favour of international academic exchange with the Soviet Union, as a means of fostering *detente* and of opening channels through which pressures can be exerted, when necessary, for the defence of academic freedom. However, the long-standing Soviet practice of sending substitutes to international conferences must inevitably tend to vitiate these advantages. The recent appeal on behalf of Leonid Plyushch, if the proposed boycott becomes a regular reaction, may put an end to the high-handed attitude of the Soviet authorities in denying visas to invited delegates. □

● News has just been received that Moscow physicist Viktor Polskii, who has been trying for four years to obtain an exit visa to emigrate to Israel, is to be brought to trial on Friday September 20, 1974. Dr Polskii who was dismissed from his post as Director of an X-ray Introspectroscopy laboratory in 1971 after applying for his visa, has several times been threatened with trial and imprisonment, if he would not withdraw his application.

In March, 1974, the authorities received an unexpected stroke of luck when a 19-year-old girl tried to com-

mit suicide by throwing herself under Polskii's car, following a quarrel with her parents. Although she and her parents at first admitted the suicide attempt, they later changed their testimony, and now claim that she simply ran across the street. Dr Polskii is therefore faced with prosecution on a charge which carries a maximum sentence of "deprivation of freedom for three years".

The trial, at first scheduled for August 15, was postponed indefinitely, on the grounds that Polskii needed an operation for hernia. There was some

speculation that this was a diplomatic retreat on the part of authorities, especially since it seemed that Dr Tsibin, who admitted the girl to hospital following the incident, was prepared to substantiate that she did, in fact attempt suicide. It was felt that the postponement would lead to the charge being quietly dropped.

This, however, is not the case. The trial is to go forward, and the timing of the announcement, during the Jewish High Holidays, seems to carry considerable overtones of propaganda to discourage others.

PROFESSOR Peleska's letter to *Nature* (July 12), a shocking document, is but one example of the great devastation of the Czechoslovak science and culture, which started back in 1969-70. It is almost impossible to collect all cases of this continuing persecution and one could hardly find a journal or editor willing and able to publish the endless lists of tragedies and frustrations of Czechoslovak scholars and intellectuals.

Professor Burhop's comments (August 9) are too mild and indecisive. No scientist is against international cooperation in science. But what meaning has such cooperation *per se* without any conditions? I am afraid that the damage caused by closed mouths and eyes (which, in fact, is the price for such unconditional "cooperation") is much greater than possible effects.

Professor Burhop reported two further cases of persecution of scientists. The first one—of Ferreiro de Alencar—belongs to cases of judicial persecution, the second—the dismissal of Iolzer because of his membership to the Communist Party—belongs to what may be called extrajudicial persecution.

To the first category of judicial persecution of scientists several examples from my country Czechoslovakia could be added. Let me give here only the names of historians Professor Hübl and Dr Tesar, and a psychologist, Professor Sabata; all these are serving sentences exceeding six years for their political activity. It will no doubt be very useful and it will certainly only increase the prestige of WFSW if they would show more initiative in collecting, publishing and condemning such cases of judicial persecution no matter where and when they may happen.

The extrajudicial persecution of scholars and intellectuals is widespread throughout the world; in my country at present it has the dimension of a national catastrophe. The numbers of Czechoslovak intellectuals fired for taking stands, for expressing and defending their opinions, who cannot work in their field (or have difficulties with their self realisation for these reasons)

are perhaps being measured now by tens of thousands—too many for a country of 14 millions which can assert itself solely by the extent to which it can contribute to the common culture and is able to help others with experience and knowledge.

I join the protests against the dismissal of Holzer. It seems to me, however, and this is not properly understood in Western countries, that his dismissal, however tragic, does not pre-

Professor F. Janouch, of the Niels Bohr Institute, Copenhagen, writes on the plight of the Czechoslovakian academic

vent him from continuing his scientific career; does not prevent him from publishing his papers or books; does not concern the very existence of himself and even of members of his family.

The extrajudicial persecution in so-called socialist countries, with the absolute monopoly of political power concentrated in one party or even in a very small group of people, with strict censorship of all mass media, with nationalisation of all branches of education, science, culture, trade, industry, administration and mass media, is incomparably more severe and effective. A man, subjected to extrajudicial persecution in these countries, has no other choice than to take unqualified, manual work. With few exceptions, which may be counted on the fingers of one hand, Czechoslovak intellectuals have no possibility of leaving the country to find work abroad. Their children are deprived the right of education at schools and universities. Their papers and books cannot be published. Let me mention here the shameful instruction issued by Czech Education Minister J. Havlín on May 19, 1972, according to which "it is not permitted to publish authors, who have been expelled from the Communist Party of Czechoslovakia, have been dismissed for political reasons from the university or emigrated ille-

gally abroad." I have tried to protest against this, in my opinion, unconstitutional and immoral instruction. The Prosecutor General of the Czech Socialist Republic did not, however, find it necessary to intervene over Havlín's instruction. Such practice, hence, has to be considered as legal in my country.

The consequence of extrajudicial persecution is the isolation from science, which is in our days of rapid development and frequent changes in all fields of science equivalent to scientific disqualification and consequent intellectual death.

Let me point out yet another aspect of Professor Peleska's case: preventing a physician from performing his profession, developing new methods for the treatment of people, easing their suffering, and saving human lives is a crime against humanity—the worse crime because it is covering itself with an ideology which pretends to be the most progressive and most humane one.

The WFSW, no doubt, is aware of the fate of one of the most renowned Czechoslovak scientists, microbiologist Academician Ivan Málek (one of the WFSW founders and for a long time its Vice-President). In autumn 1973, Professor Málek lost his position at his institute and is now being deprived of any possibility of continuing his scientific work. Even his library and his scientific archives have been thrown out of the institute, which Málek founded, built up and directed for almost 20 years. Why does the WFSW remain silent in the face of such intellectual barbarism? We are living in an open world, and only open, public stands, protests and statements are meaningful and helpful. Intellectual freedom is indivisible, and if world scientific and intellectual organisations continue their ostrich politics with respect to the fates of their persecuted colleagues, they will not only lose the moral right to speak on behalf of the world intellectual community, they will lose their intellectual freedom too.

correspondence

Whales

SIR,—With reference to the article "The Unendangered Whale" (August 9), we feel that it is most important to point out that the opinions expressed by Dr Ray Gambell are by no means universally accepted.

Gambell states that the "concept of species management is now operative". But 'concepts' do not ensure that the whales will be harvested rationally. There are many more practical factors which come into play besides scientific concepts of species management. At the International Whaling Commission (IWC) meeting last June, some of the recommendations of the Scientific Committee (of which Gambell is a member) were overruled in the plenary session in favour of higher quotas. It is also worth noting that there is still time for Japan and the Soviet Union to opt out of any of the management policies under the 90-day rule. In fact, there is no real need for Japan to opt out of any of the decisions made by the IWC as she owns dummy companies (joint ventures, flags of convenience) in countries which do not even belong to the IWC.

Gambell also points out that it also "remains to be seen just how well the scientists can resist the political and economic pressures". Past experience (of which there is much) suggests that they are unlikely to be able to resist such pressures. Some Japanese whaling scientists have admitted that they are torn between the demands of the Japanese whaling industry and the necessity for sound conservation measures.

The reason why many experts are now opting for a 10-year moratorium on commercial whaling is because they have begun to realise how little they know about whales and the effect of whaling on their populations. Gambell is not one of these scientists, but he has conceded that "there is much research needed". It has also recently been brought to Gambell's attention that perhaps the biomass of whales should be studied, not just their numbers. Had the scientists looked at the wider effects of whaling—other than those demanded by the industry-dominated IWC—they would have seen that the weight of sperm whales, for example, has declined rapidly during the past 30 years, and that in terms of biomass is probably well below maximum sustainable yield (MSY). There

is also reason to believe that the total biomass of baleen whales in the Antarctic is also well below MSY. We feel, therefore, that Gambell is rather premature in reporting that the whales, situation is satisfactory.

Fortunately the British government is still in support of the complete moratorium on commercial whaling despite Gambell's advice.

Yours faithfully,

JOHN A. BURTON

ANGELA KING

*Friends of the Earth Limited,
London W1*

Biohazards and the law

SIR,—Brian Ford (*Nature*, August 2) may be right in saying that there is not enough *statute* law about biohazards, and his suggestions for future legislation are certainly valuable. But statutory codes of practice cannot by themselves solve the problem. Sooner or later, by accident or design, someone somewhere will ignore them. What is important is what happens then.

The offender may be fined or sent to prison, but that will provide little comfort for the victim. Far more important is the civil remedy which the *common law* (i.e. the law made by the judges) has designed for such cases, centuries before Parliament started to take an interest in this field.

Under the rule known as *Rylands v. Fletcher*, anyone who for his own purposes keeps on his land anything likely to do mischief if it escapes is answerable for all the damage which is the natural consequence of its escape. The liability does not depend on negligence: all the victim has to prove is that the mischievous thing was kept there, escaped, and caused him damage, and it avails the defendant nothing to show that he took all possible care, or even that he did not know the thing was dangerous.

There are cases in the books, as recently as 1928, which apply this rule to poisonous substances. I know of no reason why the courts should not apply it to living organisms—though, if they were held to be animals, they would now come under the Animals Act 1971, which replaces common law rules broadly similar to the rule in *Rylands v. Fletcher*.

If every owner, director and operator of a laboratory which kept human pathogens on its premises realised that in the event of an escape he would be

absolutely liable to compensate all victims for all the damage they suffered, the standards of care to prevent escape from those laboratories might well be higher, and more strictly observed, than any new legislation could expect to achieve.

Yours faithfully,

PAUL SIEGHART

*Council for Science and Society,
6 Gray's Inn Square,
London.*

Megalithic alignments

SIR,—As one who has recently reviewed several of John Michell's books at some length *Men, myths and megaliths*, Thames and Hudson; in the press), I read his letter (August 23) with considerable interest.

To anyone who has made the effort to wade through the corpus of his bizarre geomancies, it comes as no surprise that most archaeologists have declined the privilege of reviewing his latest efforts. I suspect that many regular readers of *Nature* will be unfamiliar with Mr Michell's books and his ideas. Basically he is a neo-Straight-tracker, a disciple of Alfred Watkins (*The Old Straight Track*, Methuen; 1925) who believed, after a mountain-top 'vision' one summer afternoon in about 1920, that Britain was networked with alignments (including Megalithic ones) which had been laid out in premeditated fashion by some ancient race.

But Michell is also a self-confessed flying-saucer enthusiast; an admirer of Piazzi Smyth's Pyramid theories, Mrs Maltwood's Glastonbury zodiac and several other claptrap cults which provide the right kind of fuel for his own romances.

Michell's technique, rather cunningly, is to throw in any semi-respectable or respectable material in attempts to add verisimilitude to his own. For example, Fred Hoyle must be 'delighted' to find that Michell is a staunch supporter of his 'Stonehenge drum-beat theory' (about 1966). In *The View Over Atlantis* (Abacus, 1973) he writes (page 183): 'Professor Hoyle in an article in *Antiquity* suggested that the men who built Stonehenge may have communicated over long distances by beating drums . . .' and then Michell splices in his own ideas ' . . . it was with drums, songs and the clash of cymbals that magical flight was achieved according to legend.

Stone levitated by sound could become a flying chariot moving along the line of a certain magnetic intensity, whose course was marked out on the ground by alignments of stones and earthworks, linked by raised causeways and rides through the forest. With the Earth's magnetic [sic] field regulated and the streams that run through it diverted to conform to straight lines, the stone craft and its navigator could float from centre to centre, picking their way through a canal network of alternating currents and choosing the level of intensity to which their vibrations were attuned . . . ?

Geophysicists also please note!

Yours faithfully,

P. LANCASTER BROWN

Beaconsfield, Bucks

Closely observed trains

SIR,—The facile comment under the photograph on page six of your journal of the 6th of September, prompts me to raise a few points with regard to the present state of magnetic suspension technology in this country and abroad.

How good at speed? Your correspondent gives the answer herself on page seven. The 45 mile track being constructed in Germany is for a high speed system using controlled electromagnets. The proposed speeds are 500 km/h. Krauss Maffei have no problems that they cannot overcome on eddy currents up to 400-450 m.p.h. Our own tests confirm the Krauss Maffei measurements made in 1972 when they ran their vehicle at over 150 k.p.h. Nobody in their right minds and connected with the d.c. electromagnet suspension technology would see any problems at speeds as low as 50 m.p.h. The problems at Toronto are those of linear induction motors and the inverter drive for them. It is also worth noting that work on the French Aerotrain has come to a halt and discussions have taken place between the French and German authorities on a joint programme of development of the controlled electromagnet systems, for intercity and transeuropean network. So much for the misplaced Sussex claims.

There is in my opinion, an obsession with high speed and ground transport which in turn leads to a complete blinding of the technical problems (never mind the social problems) of high speed vehicles. Guidance of such vehicles is not a matter of showing that 20lb aluminium plates or one ton vehicles in laboratories have stability. The turning moments on a 30 ton, 100 passenger vehicle are of the order of 40000 N.M. The aerodynamic drag alone is more than 2000 kW and therefore that is the size of the required linear motor. (The suspension power using d.c. electromagnets is 30 kW).

No-one claims to have solved the

problem of collecting this sort of power at 300 m.p.h. from what your correspondent calls "normal a.c. mains". It is a little bit more difficult than plugging a shaver into a socket. If two of these trains cross each other this will impose an impulse load of more than four MegaWatts on the area generating board. Is the generating board going to be happy about this happening at three min. intervals? Are the high speed vehicles to run from A to B only or are they intended to change routes at speed? If yes, has some consideration been given to each proposal including the tracked hovercraft? The cost apart, these are some of the more formidable problems of high speed transportation vehicles. There are a few mundane ones like response times of the guidance systems, an accurate estimate of propulsion and suspension powers (no-one gets this as a bonus, as has been claimed), dependence of guidance and suspension forces on speed, power factors and efficiencies of the proposed equipment to name a few.

My team and I have lived with these problems for the last five years and are in a position to offer experimental evidence on suspension, guidance and propulsion aspects (including eddy current drag). We do not claim to have solved the world's transportation problems or to offer the ultimate system. The Germans have been developing their d.c. electromagnet systems for the last three years and are going ahead with the same technology for high speed application. The Japanese on the other hand have run into serious problems of guidance on their cryogenic system. We could investigate our system on the Brighton seafront to find out (a) whether it is possible to implement it in our cities and (b) whether a high speed system is feasible. Alternatively, we could always buy the finished article from Germany say, in 1985 or 1990.

The choice of Brighton seafront is not so gimmicky as it appears. Environmentally it is one of the toughest locations one could choose with corrosion and high winds presenting a severe test of any installation.

Whether Professor Laithwaite's magnetic river system is superior to all others can only be judged when he produces enough technical data and hardware to justify his claims. Even if it is, his problems will only then begin. A high speed transport system cannot be developed on a domestic market. A transeuropean system is likely to be German-French dictated. Perhaps Sussex claims are not all that misplaced!

Yours faithfully,

B. V. JAYAWANT

University of Sussex,
Applied Science Laboratory,
Brighton

Time for a change?

SIR,—In *Nature*, Vol. 250, August 30, 1974 was published a paper by Charles E. Chaffey on a time-measuring system based on SI units. Obscurantism for its own sake is admirably displayed in this paper. The time-system postulated is unintelligible, having no frame of reference, and being unrelated to reality—days, lunar months and years, which exist on this planet in the absence of all arbitrary measurement.

The author states that his aim is "the elimination of timers graduated in diverse units", presumably without regard to convenience or intelligibility. The SI second, though defined differently, is still based upon day length divided by $24 \times 60 \times 60$, therefore is irrational in terms of the decimal system (the division of the clock dial into 12 or 24 is rationally related to the geometry of the circle). Surely, if a so-called 'rational' system is to be adopted by scientists, then it should be the metric clock and the French Revolutionary Calendar (now being the last month of 182).

Incidentally, what is the starting point of C. Chaffey's calendar? The Christian era? Surely not. Why not Sir Isaac Newton's birthday? This would have the merit as being the same day as Christmas, and would remind all scientists of this great alchemist/astrologer/theologian who founded our new religion.

Yours faithfully,

NIGEL PENNICK

Trumpington,
Cams.

Group papers

SIR,—Alan Mackay's proposal (*Nature* 250, 698; 1974) that papers should be published in the name of the research groups rather than of the individuals, deserves serious consideration in spite of his outdated references (to St. Matthew and Church of England Articles). This procedure has in fact been encouraged in China. The prime purpose of scientific communication is definitely better served by an improved standard, and decreased number, of publications (have you not read any recent issue of *J. Irreg. Results?*). No author or authors of any paper can claim exclusive credit for it; why, otherwise should there always be acknowledgements? Under the new arrangement, individuals will still get the 'carrot' by belonging to a group which publishes a lot of good papers, and they won't miss the 'stick'.

Yours faithfully,

T. B. TANG

Imperial College of Science and
Technology,
Department of Metallurgy,
Royal School of Mines,
London SW7

news and views

Spectroscopy of organic molecules in solids

THE spectroscopist is interested in studying energy levels, the probabilities of transitions between levels, and the lifetimes of excited states. Because of the low volatility of most organic compounds their spectra are normally observed by using solutions in a liquid or vitreous solvent. The ultraviolet and visible absorption and emission spectra of solutions are, however, generally very broad and the structure that may arise from molecular vibrations is absent. This loss of information can seriously reduce the value of observations of the electronic spectra of organic molecules.

In a dilute vitreous solution, the interaction of a solute molecule with its local solvent environment perturbs the energy levels of the solute. This interaction is different at different sites in the glass so the sample comprises a range of distinguishable solute molecules; the spectrum of such a sample is said to be 'inhomogeneously broadened'. In a liquid solution, all solute molecules experience the same environmental influences (when an average is taken over the time that the molecule exists in the particular energy level) and the spectrum is 'homogeneously broadened'.

The absorption spectrum of a glassy solution is broad, even at very low temperatures when the vibrational motion of the solvent is suppressed. Its emission spectrum is also broad when the excitation is due to a broadband arc source. The emission, or fluorescence, spectrum is caused by transitions from the lowest vibrational level of the electronically excited molecule to various vibrational levels of the ground electronic state (higher vibrational states of the electronically excited molecule generally decay very rapidly by non-radiative mechanisms to the lowest level, so the vibrations of the excited molecule are not normally seen in emission). Vibrational structure is not seen in such an emission spectrum if the variations in the energy-level differences due to site changes are larger than the vibrational spacings, and this is the usual situation for organic species.

On page 215 of this issue of *Nature*, Eberly, McColgin, Kawaoka and Marchetti of the University of Rochester and the Eastman Kodak Research Laboratories in Rochester, New York, describe a method for obviating the effects of inhomogeneous broadening of the electronic spectra of organic solutes in vitreous solvents. They employ a narrow-band laser source and observe vibrational structure in emission spectra. Their success depends on the fact that only a small fraction of the solute sites in the glass is in resonance with the narrow-band source, leading to a selection of a particular class of solute molecules (those whose environments are sufficiently similar to let them absorb the incident radiation). Their technique is particularly effective when the incident radiation has the frequency corresponding to the energy difference of the lowest vibrational levels of the excited and ground states, the 0-0 band. At higher frequencies, variations in the site may bring different vibrational levels of the electronically excited molecule into resonance. Actually a number of "extra" emission lines were seen from a 10^{-4} M solution of phenanthrene in a glass at 4.2 K when the incident frequency was 0.24 eV ($1,900\text{ cm}^{-1}$) above the centre of the 0-0 absorption band.

The successful elimination of inhomogeneous broadening

of the spectra of dilute solutions of organic molecules in glasses is reminiscent of the Lamb-dip, of a sharp form of two-photon absorption in the high-resolution spectroscopy of gases, and of the spin-echo experiment in nuclear magnetic resonance. The Lamb-dip and sharp two-photon absorption may be seen in gases in intense radiation in a standing wave, when only those molecules with negligible velocity in the direction of propagation interact with both the forward and reflected beams; Doppler broadening is thus eliminated. Hahn's spin-echoes arise through a reversal of the precession of magnetic moments through the application of a second pulse; the magnetic field inhomogeneities speed up or slow down both the forward and reverse precessions, leading to a coincidence, or 'echo', in the detector and the elimination of the broadening due to field inhomogeneities.

The Rochester experiments have yielded structure in the emission spectrum of naphthacene in a glass at 4.2 K. This structure has been analysed to give vibrational frequencies of the molecule in its ground electronic state. Though this is interesting and promising, and probably capable of wide application because of the availability of narrow-band tunable dye lasers, it should not be forgotten that these frequencies are easily obtainable from infrared and Raman spectroscopy.

DAVID BUCKINGHAM

Nude mice

SINCE their original description in 1966 (Flanagan, *Genet. Res.*, **8**, 295), nude mice have spread through the immunological world at a remarkable pace. While mice heterozygous for the mutant nude gene (*nu/+*) are apparently normal, homozygotes (*nu/nu*) are hairless (hence their name) and have hypoplastic thymus glands (Pantelouris, *Nature*, **217**, 370; 1968). The thymus abnormality leads to a marked deficiency in thymus-derived (T) lymphocytes and nude mice are rapidly replacing thymectomised mice as models of T cell deprivation and as a source of relatively pure B lymphocytes for *in vitro* studies. In many laboratories the *nu* gene is being back-crossed onto a variety of inbred backgrounds thereby increasing their usefulness for cell and tissue transfer experiments. The T cell deficiency in nudes make them highly susceptible to infection (but apparently not to cancer) and they survive poorly in conventional animal houses. Thus they are usually produced by mating heterozygotes.

The extent of the T cell deficiency in nudes has remained controversial. They are usually referred to as being 'congenitally athymic' but this is clearly a misnomer in view of the presence of a thymus gland in all nudes so far studied. They have been shown to have T cell precursors which can repopulate a normal thymus (Wortis *et al.*, *J. exp. Med.*, **134**, 681; 1971) and develop into immunocompetent T lymphocytes (Kindred and Loor, *J. exp. Med.*, **134**, 681; 1971) and several reports have suggested the possibility that small numbers of T cells are present in at least some nudes. For example, approximately 1% of lymphoid cells in nude spleen and lymph nodes label directly with fluoresceinated alloantibodies directed against the θ (thy-1) alloantigen, a T cell marker in mice (Raff, *Nature*, **246**, 350; 1973). Although θ is expressed on some neural cells, epidermal cells, and fibroblasts, in addition to thymus and T lymphocytes, there is no evidence that it is

found in readily detectable amounts on B lymphocytes, granulocytes, macrophages or other cells in lymphoid or haemopoietic tissues (Owen *et al.*, *Nature*, **249**, 361; 1974). Thus it seems likely that these few θ^+ lymphoid cells in nude are T lymphocytes, although their origin (host or maternal, thymic or extrathymic) and functional capabilities remain unclear.

On page 229 of this issue of *Nature*, Loor and Roelants report the presence of another population of θ^+ cells in nude spleen which are distinguished from normal T lymphocytes (and those described previously in nudes) by having such small amounts of θ on their surface that they can be detected only by indirect immunofluorescence, particularly with an absorbed rabbit anti-mouse brain serum which appears to have anti- θ -like specificity. These cells lack detectable surface immunoglobulin (a marker for B lymphocytes) and make up approximately 20% of nude spleen cells, but are present in very small numbers in nude lymph nodes and in lymphoid tissues of normal mice. The identity of these cells is unknown and it seems premature to refer to them as T cells, which implies their migration from the thymus. Loor and Roelants make the intriguing

suggestion that these cells may be precursors of T cells, which perhaps have followed some aberrant differentiation pathway due to the lack of a normal thymus. This is an attractive possibility in view of the recent evidence that one can induce the appearance of θ and other thymus alloantigens in cells lacking readily detectable amounts of these antigens by exposing them to a variety of substances (such as dibutyl cyclic AMP and thymus extracts) for 1–2 h *in vitro* (Scheid *et al.*, *J. exp. Med.*, **138**, 1027; 1973).

Many pieces still are missing in the nude puzzle, the most important being the role of the presumptive wild-type protein in normal thymus and hair development. The extent of the T cell deficit and the origin and nature of the θ^+ cells in nude lymphoid tissues may be answered, in part at least, by studying nude mice born in *nu/nu* parents, rather than of *nu/+* parents as has been the case thus far. Only in this way can the role of maternal and normal litter-mate T cells and thymus humoral factors on nude embryos be determined. If such mice lack θ^+ cells in their lymphoid tissues it would establish once and for all the essential role of the normal thymus in their production.

MARTIN C. RAFF

When the ice age seemed to end

IN the opening years of this century a sequence of muds and clays exposed in the Island of Sjaeland, Denmark was described. In two clay layers was an abundance of remains of the arctic alpine plant, *Dryas octopetala*, considered to be indicative of cold conditions. The intervening mud contained remains of a more temperate flora including birch trees. The sequence was thought to represent a climatic oscillation at the close of the last (Weichselian) glaciation, and the temperate interstadial was termed 'Allerød' after its site of origin. Subsequently this sequence was found over a wide area of north-west Europe and the advent of radiocarbon dating has made it possible to assign tentative dates to the lithological boundaries. West (in *Pleistocene Geology and Biology*, Longman, London; 1968) quotes the following values, which are averages of many dates:

Older Dryas/Allerød Interstadial	11,950 b.p.
Allerød Interstadial/Younger Dryas	10,750 b.p.
Younger Dryas/Preboreal	10,250 b.p.

Further investigations in Denmark, however, led to complications in this basic scheme. At Bøllingsø a sequence was found in which the clays of the Older Dryas period were interrupted by a mud layer, indicating an interstadial predating the Allerød. Similar sequences have now been found in Germany, Holland and Norway, and several radiocarbon dates are available. Van der Hammen *et al.* (*Geologie Mijnb.*, **46**, 79; 1967) date the episode at 12,400–12,000 b.p. in the low countries, but a Norwegian date (Chanda, *Årbok Univ. Bergen*, No. 1, 1; 1965) places its openings at 12,650 b.p. In some British pollen diagrams there is evidence of a minor vegetational change in pre-Allerød times, for example at Haweswater (Oldfield, *New Phytol.*, **59**, 192; 1960) and, more convincingly, at Tadcaster, Yorks. (Bartley, *ibid.*, **61**, 277; 1962) but neither site has been radiocarbon dated.

A kettlehole site, now beneath Blelham Bog near Windermere, was found to have two organic layers in its late Devensian (≡late Weichselian) sediments. Pennington's pollen diagram (in *Studies in the Vegetational History of the British Isles*, edit. by Walker and West, Cambridge; 1970) of these layers shows a proportional increase in tree birch pollen during the lower organic layer, but the radiocarbon date obtained (14,330±230 b.p.) is considerably older than the classic Bølling interstadial. Subsequently Pennington and Bonny (*Nature*, **226**, 871; 1970) have confirmed the dating of this layer with a further date of 14,280±230 b.p. and have also clarified the vegetational events of this period by means of absolute pollen sedimentation determinations

(see Davis and Deevey, *Science*, **145**, 1293; 1964). They found that the pollen input rate of the lower organic layer was similar to that in the adjacent clays; the apparent rise in birch pollen was a statistical artefact resulting from the use of proportional expressions. Thus in Britain there is still no well authenticated and dated interstadial equivalent to the continental Bølling which is separable and distinct from the subsequent Allerød interstadial. Bartley's Tadcaster site remains the most hopeful contender.

At Colney Heath in Hertfordshire there is a peat which may have been transported by a glacier and buried in detritus. Godwin (*Proc. R. Soc.*, **B150**, 258; 1964) described an arctic flora from this peat and dated it at 13,560±210 b.p. This material thus postdates the Blelham lower organic layer, but gives no indication of an improvement in climate.

Datings of the opening of the supposed Allerød interstadial have usually depended upon lithological changes, in particular the replacement of minerogenic sediments by organic ones. Early dates of this boundary showed considerable consistency and were also in close agreement with continental dates. Godwin and Willis (*Proc. R. Soc.*, **B150**, 199; 1959) quote such dates as 11,942±120 b.p. (Lunds, Yorks.), 11,880±225 b.p. (Keith, Banffs.) and 11,870±120 b.p. (Windermere). Subsequent dates have not shown this degree of consistency, for example at Loch Droma, Ross and Cromarty, a date of 12,810±155 b.p. was obtained (Kirk and Godwin, *Trans. R. Soc. Edinb.*, **65**, 225; 1965). This date was very difficult to explain since it predated even the Bølling and yet gave all indications, lithological and palynological, of being the commencement of the Allerød. Other similar dates have now been obtained from a variety of sites, particularly in Scotland. Bishop (*Trans. J. Proc. Dumfries Galloway nat. Hist. Antiq. Soc.*, **40**, 117; 1963) gave a date of 12,940±250 b.p. to the opening of the interstadial at Lockerbie, Dumfries; Sissons and Walker (*Nature*, **249**, 822; 1974) give dates of 12,750±120 b.p. for kettle hole deposits near Callander, Perth, and 11,290±165 and 13,151±390 b.p. for Loch Etteridge in the Grampians of Scotland. At the latter site, the younger date corresponds to the commencement of gyttja sedimentation and the older date refers to the underlying clay gyttja. Pennington and Bonny date the climatic improvement (as evidenced by a considerable increase in absolute pollen influx) in Blelham Bog at between 12,700 and 12,950 b.p. Simpkins (*New Phytol.*, **73**, 605; 1974) has recently published a date of 12,050±250 b.p. for the base of organic sediments from a kettlehole at Glanllynau,

North Wales, but Coope and Brophy (*Boreas*, 1, 97; 1972) have obtained a date of $12,556 \pm 230$ b.p. for the same layer using only the macrofossil fruits of cyperaceous plants for the dating and thus avoiding contamination from penetrating roots. At Glanllynau, the basal organic sediments contain little tree birch pollen, the expansion of this constituent occurring 10 cm above the clay/gyttja transition. Juniper pollen is poorly represented throughout, but there is a trace of expansion within these basal layers. An analysis of the fossil Coleoptera of this site by Coope and Brophy gave evidence of climatic improvement well below the lithostratigraphic change, at $12,850 \pm 250$ b.p. This improvement was reflected neither in the lithology nor in the relative proportions of the pollen assemblage at that level.

This collection of dates and observations is leading some researchers, particularly Pennington, to propose that some of the lithological and biological changes which have been taken to represent the commencement of the Allerød interstadial, *sensu stricto*, are in fact indicative of the climatic amelioration which gave rise to the Bølling sediments on the continental mainland. Climatic improvement may have begun by 13,000 b.p. but lithological changes in some sites was delayed for a thousand years. In Britain the two interstadials seem to have run into one, particularly in the northern and western parts of the islands. Presumably the oceanicity of the British Isles has influenced the degree to which climatic thresholds have been successively crossed; as a result, the response of the biota and any fluctuations in the nature of sedimentation have been dampened in the amplitude of their oscillations. It would seem that the search for a Bølling in the west of Britain is at an end.

Faunal evidence for a decline in temperature during the interstadial has been found as early as 12,000 b.p. by Coope *et al.* (*Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 10, 87; 1971). Lithological evidence for the close of the interstadial and the onset of solifluction is dated $10,828 \pm 185$ b.p. at Scaleby (Godwin, Walker and Willis, *Proc. R. Soc.*, B147, 352; 1957) and at Loch Etteridge, Sissons and Walker obtained a date of $10,765 \pm 120$ b.p. The final close of the Allerød thus seems to be a well marked occurrence, the date of which may vary slightly from one locality to another dependent on local conditions such as relief.

The end of the cold Younger Dryas period was dated by Godwin *et al.* to $10,257 \pm 350$ b.p. at Scaleby Moss, but there is a problem of lithological and biological definitions of this boundary. At Scaleby the date refers to the first rise in the proportion of juniper pollen.

Some other dates for this change are $10,490 \pm 160$ (Blelham, Pennington and Bonny), $9,590 \pm 170$ (Bigholm Burn, Dumfries, Moar, *New Phytol.*, 68, 433; 1969), $9,508 \pm 200$ (Red Moss, Lancs., Hibbert *et al.*, *Proc. R. Soc.* B177, 161; 1970) and $9,660 \pm 105$ (Slieve Gallion, Co. Tyrone, Pilcher, *New Phytol.*, 72, 681; 1973). As Smith and Pilcher (*New Phytol.*, 72, 903; 1973) have pointed out, this biologically defined horizon is evidently diachronous within the British Isles. The use of lithological features (that is, the replacement of clay by primarily organic sediments) is equally variable, however. Birks (in *Past and Present Vegetation of the Isle of Skye*, Cambridge; 1973) gives dates of $9,420 \pm 150$, $9,482 \pm 150$ and $9,655 \pm 150$ b.p. for the base of Flandrian muds in Skye. Sissons and Walker also have a late date, $9,405 \pm 260$ b.p. for a similar horizon at Loch Etteridge. Evidently the conditions necessary for lithological change were satisfied at different times in different parts of the country, depending upon local climate, relief, geology and so on. The definition and dating of the close of the late-Devensian period and the opening of the Flandrian thus remain rather obscure.

With the increasing number of radiocarbon dates and biological and lithological analyses of late-Devensian deposits in Britain, the complex underlying climatic changes within this unstable period are beginning to be understood. It is becoming clear that simple threefold division of the period is a gross and rather misleading misrepresentation of the diverse temporal and spatial conditions found in the British late-Devensian. PETER D. MOORE

New Miocene polarity scale

from Peter J. Smith

THE statistical analysis of geomagnetic field reversals, begun by Cox (*J. geophys. Res.*, 73, 3247; 1968) and later developed further by Blakely and Cox (see, for example, *J. geophys. Res.*, 77, 4339; 1972), seems a complicated business to non-mathematicians. Fortunately however, most of the conclusions emerging from it are relatively easy to understand and offer an interesting example of the predictive power of statistics where a data set is incomplete and likely to remain so for some time. The problem with the data set in this case is that, although relatively long periods of single polarity may be detected and measured with reasonable accuracy, the lengths of very short polarity intervals lie within the errors of the dating technique. This, coupled with the fact that short intervals are intrinsically unlikely to be represented by very many magnetised rock samples,

makes it difficult to investigate experimentally any variations in field behaviour (for example, reversal frequency) with time.

Previous statistical work on the geomagnetic time scale has indicated that field reversals apparently occurred randomly throughout the Cainozoic and may be described by a Poisson probability model with an average reversal frequency λ . But λ seems to vary with time, most markedly during the Miocene. The frequency of known reversals over the past 45 million years (Myr), for example, was 3.0 Myr^{-1} , whereas the period 22.7–7.3 million years ago had an anomalously low average reversal frequency of 2.5 Myr^{-1} . So the question is: did the reversal rate really decrease over this limited period, or do other reversals (probably linked with short polarity events) remain to be discovered?

To find out, Blakely (*J. geophys. Res.*, 79, 2979; 1974) has analysed 18 total field profiles from the north-east Pacific, the east Pacific rise and the Indian–Antarctic ridge using the signal-averaging technique previously developed by Blakely and Cox, a technique by which profiles are combined and normalised to a common average spreading rate. Among the important results to emerge or follow from this analysis are the following:

A comparison of the combined (stacked) profile from 14 north-east Pacific profiles with a computer-generated model profile from the time scale of Heirtzler *et al.* (*J. geophys. Res.*, 73, 2119; 1968) shows that for the period 22.7–7.3 Myr there are eight short-wavelength magnetic anomalies present which are not predicted by the previously published time scale. In particular, anomaly 5, originally thought to be a single constant polarity epoch lasting for 1.15 Myr, is now seen to comprise five short-wavelength anomalies, thus confirming the result of Cande and Labrecque (*Nature*, 247, 26; 1974). Additional data from four east Pacific rise and Indian–Antarctic ridge profiles (areas distant from the north-east Pacific) confirm the presence of the eight extra anomalies on a worldwide basis.

A plot of average spreading rate for the 14 principal profiles taken together (constructed from data obtained incidentally in the main analysis) suggests that the rate varied often, and often quite violently, over the period 22.1–7.6 Myr ago. Between 19.3 and 18.5 Myr ago, for example, the spreading rate apparently decreased from 47.7 mm yr^{-1} to 21.8 mm yr^{-1} —a surprising deceleration within only 0.8 Myr. Apparent spreading rates for the other two areas of study taken separately fluctuate with time in a similar manner, implying further that spreading rates were

changing coherently throughout the world. The face-value interpretation of these results is that, during the Miocene, seafloor spreading did indeed proceed discontinuously.

There is another possible explanation, however. The basis of comparison used by Blakely in his analysis was the time scale of Heirtzler *et al.*, a scale which assumes that seafloor spreading was constant at the mid-Atlantic ridge during the Cainozoic and which used the single magnetic profile from Vema cruise 20 in the South Atlantic. But this interpretation may not be valid. If, instead, it is assumed that the Pacific, Indian and Antarctic plates were each moving uniformly during the Miocene, then the Heirtzler scale must be adjusted. For various reasons (for example, that accelerations of entire plates relative to the asthenosphere would have had to have been improbably high at 20 km Myr⁻² or even 40 km Myr⁻²) Blakely concludes that such uniformity is likely, or at least the most likely situation given current data. As Blakely points out, although many examples of variable spreading rates have been described by other workers, most are from localised areas and do not include evidence for variations synchronised on several plates. The assumption of zero acceleration for plates is thus the simplest, but still "tenuous at best".

Nevertheless, using this assumption Blakely goes on to construct a new Miocene time scale which adds 18 reversals to the Heirtzler time scale for the period 22.7–7.6 Myr. Sixteen of these reversals are derived from the eight short-wavelength anomalies, and the remaining two are obtained by dividing the positive interval at 13 Myr on the Heirtzler scale into two smaller positive events. The revised scale now contains 14 short events lasting less than 0.1 Myr. It also has an average reversal frequency of 3.7 Myr⁻¹, which is a significant increase on the previous 2.5 Myr⁻¹. At the same time, the new figure is higher than the 3.0 Myr⁻¹ rate derived from known reversals over the past 45 Myr, which suggests that there may also be some undetected events outside the more limited Miocene period considered by Blakely.

Finally, by comparing magnetic profiles for crustal magnetisation models with real profiles, Blakely is able to estimate the width of the transition zones from one polarity to the other. In each of the three models concerned the magnetised crust is regarded as a 0.5 km thick horizontal slab with its upper boundary at a depth of 3.5 km, but the models differ in the width of the band about the spreading centre over which new crust becomes magnetised. The best fit to the data from

the north-east Pacific is obtained for a model in which the transition between polarities takes place over a horizontal distance of 6 km. This figure is, however, highly dependent on the thickness of the magnetic layer; for a layer thicker than 0.5 km, 6 km would be an overestimate.

Anti-actin

from Dennis Bray

THE rumour that antibodies cannot be produced against actin lasted about five years. Born of one or two unsuccessful attempts to raise antisera to this major protein of muscle, the rumour was nurtured by a familiar process of *post hoc* rationalisation. Actin is non-antigenic it was said, because it has such a strongly conserved structure that the small differences between species are not able to provoke an immune response. Actin is non-antigenic because it is present in almost every cell of the body so that all animals will have innately acquired tolerance. Anybody thus dissuaded from an attempt to make this most useful antiserum, however, has cause for regret. For there are now three independent reports of its production.

The starting point for two of the reports was the observation that patients with certain aggressive forms of hepatitis have autoimmune antibodies in their circulation. Although it was known from work with fluorescent antibodies that these were directed against smooth muscle, the precise nature of the antigens was not known. Gabbiani and colleagues (*Am. J. Path.*, 72, 473–488; 1973) have now examined this by absorption studies and have found that this serum cross reacts with the actomyosin of blood platelets and with the purified component known in platelet circles as thrombosthenin A, but elsewhere simply as actin. Concluding from their tests that the serum is essentially anti-actin, the authors used it in immunofluorescent tests to show the distribution of this protein. Many non-muscular tissues, including liver cells, brush borders and intestinal epithelial cells, were found to be specifically stained.

Stimulated by the same medical findings, and in the laboratory in which they were originally made, Trenchev and colleagues (*Clin. exp. Immun.*, 16, 125–136; 1974), sought to compare the autoimmune sera with those against various protein components of human smooth muscle. These were prepared by conventional methods against muscle myosin, tropomyosin and actin, and used in immunofluorescent tests. Once again a constellation of tissues were stained and a correlation between staining and the presence of microfilament arrays was noted; as in the networks of

stained filaments seen in HeLa cells. The different types of artificially induced antibodies gave slightly different staining patterns but none seemed to resemble the hepatic sera more closely than the others.

Quite a different method and a highly original one was used by Lazarides and Weber (*Proc. natn. Acad. Sci. U.S.A.*, 71, 2268–2272; 1974). Rather than prepare antisera to conventionally prepared actin, with its persistent complement of other proteins such as tropomyosin, these authors used the bold and very direct approach of injecting material recovered from SDS (sodium dodecyl sulphate) gels. The source of their material, mouse fibroblasts, is rich in actin and even a crude fractionation is adequate to give a band on acrylamide gels which is fairly pure. The denatured protein extracted from the gels is apparently a potent antigen and able to induce antibodies which cross react with both fibroblast and muscle actin. When used to stain the fibroblasts by the indirect fluorescent method this serum gave striking results. Brightly stained filaments in a wide variety of patterns are seen, sometimes aligned in parallel with the long axis of the cell or close to the outer surface, at other times arranged radially around central focal points.

Taken together these three reports provide good evidence that antibodies to actin can be produced, although the legally minded will not be without reservations. In not every case was the purity of the actin adequately monitored—a boring point but important in the light of previous experience—nor were all of the immunological controls carried out. Surprisingly, not one of the antisera was tested against adult skeletal myofibrils which surely would give a direct test of their specificity. And the use of antisera to denatured proteins is an uncertain business which, again to invoke rumours, may not have such a high degree of specificity as one is used to with native proteins.

But these are quibbles which will no doubt be resolved in time. In general the results are most encouraging, both from a medical point of view as well as that of cell biology. The technique of Lazarides and Weber, using the combination of immunofluorescence and SDS gels would seem to be of particular potency. One of its strengths is its applicability to structural proteins normally classed as insoluble and almost the first question about such proteins is: where are they in the cell? Electron microscopy can give some indication, but is limited in its ability to identify different chemical types and in the area of the cell which it can cover. Methods involving antisera and the light microscope are potentially free of both of these restrictions.

Two-nucleon transfer between heavy ions

from P. E. Hodgson

THE theoretical analysis of reactions between heavy ions in which two nucleons are transferred from the projectile to the target has now been developed to a high degree of sophistication. The basic distorted wave theory was formulated many years ago and successfully applied to analyse (d,p) reactions. But in the case of heavy ion reactions many of the approximations that are made for (d,p) reactions are no longer valid, in particular the use of a zero-range interaction and the neglect of recoil. Inclusion of these effects is very complicated and greatly increases the computation time. Furthermore it has become increasingly clear that in many transfer reactions involving collective nuclei the contributions from two-step processes that take place through the pre-excitation of the target nucleus or by post-excitation of the residual nucleus must also be included. Lastly in heavy ion reactions there are substantial contributions from a large range of incident orbital angular momenta.

All these factors have combined to make the whole calculation prohibitively long, even on the fastest computer,

if it is carried out in the usual way. Recently, however, Low and Tamura (*Phys. Lett.*, **48B**, 285; 1974) have developed new techniques for evaluating the multidimensional integrals that occur in these calculations, and these have so strikingly reduced the computing time that these calculations are now practicable.

These new computing techniques have been applied by Tamura, Low and Udagawa (*Phys. Lett.*, **51B**, 116; 1974) to calculate the cross section for the two nucleon transfer reaction $^{76}\text{Ge}(^{16}\text{O}, ^{14}\text{C})^{78}\text{Se}$ to the ground and first excited (2^+) state of the final nucleus. Comparison with the measurements of Lemaire, Mermaz, Sztark and Cunsolo shows that the distorted wave calculations are inadequate, even when finite range effects are taken into account (see dotted line in figure). The cross section for the reaction to the ground state shows too much oscillation and is too high at small angles, while that of the reaction to the 2^+ state at 0.613 MeV is far too small.

They then took into account the inelastic excitation in the exit channel. The residual nucleus ^{78}Se is easily excited into low-lying collective states, so it is possible for the two transferred nucleons to be first added to the target to give ^{78}Se in its ground state, and then for the outgoing ^{14}C nucleus to interact again and excite the ^{78}Se nucleus to the 2^+ state. This two-step contribution to the reaction adds to the direct contribution when the two nucleons are transferred so as to produce the ^{78}Se nucleus immediately in its 2^+ state. There is also a small effect on the cross section of the reaction to the ground state, for this similarly has a two-step component that goes first to the 2^+ state, which is then de-excited by inelastic scattering in the exit channel.

This complicated calculation can be carried out without any adjustment of parameters. The wave function of the two transferred protons to be added to the target was obtained using the standard Bardeen-Cooper-Schreiffer method with the Random Phase Approximation. The single-particle energies were taken from previous work and the strength of the pairing interaction was determined by fitting the experimental separation energy. The separation of the two protons from the ^{16}O was calculated using the fractional parentage coefficients of Cohen and Kurath.

The results of this calculation, taking the two-step processes in the exit channel into account, are shown by the full line in the figure. The agreement is excellent, indicating that the essential physics of the process is now well under-

stood. In particular, it is clear why the inclusion of the two-step processes produces such a dramatic improvement. Since the ^{78}Se and ^{76}Ge nuclei are both superconducting, the transition from the ground state of the target to the ground state of the final nucleus is much stronger than the transition to the first excited state. Thus inclusion of the two-step process greatly enhances the 2^+ cross section but has a much smaller, though still important, effect on the reaction to the ground state.

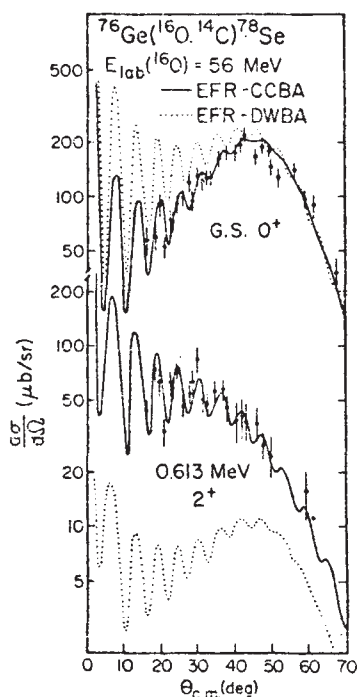
There are still more effects that could be taken into account, in particular the inelastic effects in the entrance channel. In the calculation of the form factor of the transferred protons only the most important term, corresponding to 0s relative motion of the two protons, was taken into account, and a fuller calculation could investigate the contribution of the higher order terms. Nevertheless, the success of the present calculations suggests that the most important effects have already been included, and that these improvements will not substantially affect the detailed understanding of this reaction that has now been achieved.

Gels and chains

from a Correspondent

It has been known, since the work of the Singers and of Allison in the 1950s, that gel formation in solutions of deoxy sickle-cell haemoglobin (Hb-S) is highly dependent on Hb-S concentration and that the minimum gelling concentration (MGC) is a function of temperature, pH, ionic strength and the presence of organic phosphates which bind preferentially to deoxy β -chains. More recent studies by Bookchin, Nagel and Ranney on mixtures of deoxy Hb-S with other haemoglobins showed that the MGC values were higher, but that the partial concentrations of Hb-S at gelation were lower, indicating the incorporation of other haemoglobin species in the gel. Following an earlier analysis of these results by Minton on the basis of a two-step aggregation process, they have now been re-interpreted by Moffat (*Science*, **185**, 274; 1974), assuming the formation of hybrid tetramers formed by association of unlike dimers in such binary mixtures, for example $\alpha^2\beta^A\beta^S$ from $\alpha^A\beta^A$ and $\alpha^A\beta^S$ in a mixture of Hb-S and normal adult Hb-A.

According to Moffat all possible tetramer species are in principle capable of incorporation into the gel: the gel forms when the weighted sum of their concentrations reaches a critical value, the MGC for pure deoxy Hb-S under the same conditions, which is assigned



Differential cross section for the two-proton transfer reaction $^{76}\text{Ge}(^{16}\text{O}, ^{14}\text{C})^{78}\text{Se}$ to the ground and 2^+ excited state of ^{78}Se compared with exact finite range calculations. The dotted line shows the one-step distorted wave calculation (EFR-DWBA) and the full line shows the coupled channels calculation (EFR-CCBA) that takes into account the contributions of two-step processes in the exit channel.

a weight of unity. The weights (w) can be regarded as relative association coefficients for the addition of particular tetramer species, hybrid or symmetrical, and deoxy or liganded, to the haemoglobin polymer. Since, however, deoxy tetramers have extremely small dissociation rate constants, hybrid formation is rapid in mixtures in the oxy form (as used by Bookchin and co-workers), and subsequent deoxygenation effectively freezes the resulting equilibrium mixture of tetramer species. From the value of the identical dissociation constant found for separate and mixed oxy Hb-A and Hb-S, Moffat evaluated individual tetramer concentrations and hence derived weighting coefficients for the tetramer components of the mixtures under consideration. The structural implications of these weights are clearly complex, and no clearcut generalisations emerge. For deoxy foetal haemoglobin (Hb-F) w is about 0.1, a result of some clinical interest in view of the proportions of Hb-F found associated with Hb-S in some clinical conditions. The weights do not depend greatly on the ligation state of the non-S chains, for example deoxy Hb-A and cyanmet Hb-A both have weights of about 0.5. The high w values for hybrid tetramers are also noteworthy. From the w values for deoxy Hb-A and deoxy Hb-S Moffat predicted that mixing in the deoxy form, with no hybrids present, would result in a higher MGC than mixing in the oxy form, facilitating hybridisation, followed by deoxygenation. This prediction was confirmed experimentally over a wide range of fractional composition. Moffat regards gelation as a highly cooperative process in contrast to Minton, who envisaged a two-step model in which linear polymerisation of haemoglobin tetramers into filaments is followed by lateral aggregation of the filament to form the gel.

An explanation at the molecular level for the depression of α or β chain biosynthesis in the respective thalassaemic states must be looked for by the study of gene expression and protein synthesis. Some progress has been made in elucidating the molecular basis of two forms of α -thalassaemia. Clegg and Weatherall (*Lancet*, ii, 133; 1974) now suggest that increasing circumstantial evidence for the relative instability of the messenger RNAs coding for the δ -chain of human Hb-A₂, and for the various $\delta\beta$ (Lepore) and $\beta\delta$ (anti-Lepore) chains, may be relevant to the molecular defect in some β -thalassaemias. Both Nance and Stamatoyannopoulos and coworkers have previously considered the possible role of fusion genes, of the Lepore type, in β -thalassaemia. Clegg and Weatherall now suggest that B⁰ thalassaemia, in

which there seems to be total absence of β chains, may be associated with a δ -like pattern of β -chain synthesis. In Nance's model this would involve silent Lepore-type fusion genes coding for normal β -chains, but controlled like a δ -chain gene. Clegg and Weatherall suggest that the instability of δ -chain mRNA is due to loss of stabilising regions at the 5' and 3' ends, subsequent to the evolutionary divergence of the β and δ chain genes. They discuss possible tests of their hypothesis, one of which would be the detection of small proportions, say 1%, of Hb-A in untransfused homozygotes for β -thalassaemia, containing normal β -chains but formed only in early nucleated erythroid precursors by the unstable mRNA.

Photographing a superfluid vortex

from P. V. E. McClintock

It has long been realised that superfluid helium cannot rotate bodily: it is believed that, instead, rotation of the container may result in the formation of one or more individual quantised vortex lines. Particular interest therefore attaches to a report by Williams and Packard of the University of California at Berkeley in a recent issue of *Physical Review Letters* (33, 280; 1974) that they have been successful in obtaining photographs apparently showing for the first time the positions of individual vortex lines in superfluid ⁴He.

The linear vortex, a classical phenomenon which was investigated in great detail by Lord Kelvin, consists of a cylindrical core which may or may not be hollow and, surrounding it, rotating fluid whose tangential velocity v_s is inversely proportional to the distance from the centre of the core. The vortex which forms above the plug hole as water flows out of a bath is a familiar example of a hollow cored vortex.

Vortices in superfluid helium are, however, of special interest because they are quantised: the quantity $\oint \mathbf{v}_s \cdot d\mathbf{l}$, evaluated around any closed loop in the superfluid and known as the circulation, is always equal to $n(h/m)$ where h is Planck's constant, m is the mass of helium atom, and n may be zero (no vortex core present inside the loop) or a positive integer. The flow of superfluid around the core can be described by a macroscopic wave function and occurs without dissipation of energy, much like an electron in a stable state around the nucleus of an atom, so that the existence of quantised vortices may be regarded as a macroscopic mani-

festation of quantum mechanics.

Thus, when a container of superfluid, initially at rest, is rotated sufficiently slowly, the superfluid will always remain at rest relative to the fixed stars, no matter how long the experiment is continued. If the angular velocity is increased it will, however, eventually become energetically favourable for one or more vortex lines to be created in the liquid. At large rotational speeds many vortex lines are present and the resultant motion of the liquid approximates to solid body rotation at the same angular velocity as that of the container. It had been widely assumed that the vortex cores which in helium are approximately of atomic dimensions, would all be arranged parallel to the axis of rotation and spaced in a triangular lattice relative to each other. But despite extensive experimental evidence both for the existence of vortex lines and also for their quantisation, nobody had previously been able to determine experimentally their actual positions and orientations in the container, and it is this which Williams and Packard have now accomplished.

In essence their technique was extremely simple and exploited the fact that when free electrons are injected into liquid helium they form relatively large bubble-like structures of radius about 16 Å. These negative ions, by virtue of the reduction in energy arising from the excluded rotating superfluid, are very strongly attracted towards the core of a vortex. Thus, by using a radioactive electron source and a suitable arrangement of electric fields, it was possible to charge the array of vortices inside a rotating container of superfluid helium so that each vortex had negative ions spaced out at intervals along its core, rather like a string of beads. Then, by an appropriate reversal of electric field, the ions could all be shot off the vortex lines and out through the surface of the liquid. By positioning a scintillator above the surface it was possible to ensure that the current pulse arising from each vortex line would give rise to a spatially separate flash of light, thus indicating the position of that vortex in the liquid.

In practice, despite its apparent simplicity, the experiment has proved to be considerably more difficult than was the equivalent experiment to demonstrate the lattice of vortices in a superconductor. To achieve their successful result, the authors had to perform the not inconsiderable technical feat of mounting a complete ³He-⁴He dilution refrigerator, capable of reaching temperatures below 0.1 K, on a rotating table; it had been necessary to add a small proportion of ³He to the superfluid ⁴He in order to stabilise

the vortex array, a magnetic field was required to focus the pulses of electrons leaving the liquid, and a very high quality optical system, using fibre optics to bring the feeble flashes of light up from the cold end to an image intensifier at room temperature, had to be developed. The light from the image intensifier passed into a wide aperture camera loaded with high speed film.

It was found that, with the apparatus in rotation, a series of spots did indeed appear on the film whereas, at rest, a completely blank negative resulted. The density of spots was roughly equal to the expected density of vortex lines in the liquid, and was proportional to the angular velocity.

It turned out, however, that the vortex lines were not in fact arranged in a regular manner, and moreover they appeared to wander around in the liquid in a random manner. The reason for this is not yet clear but, now that a successful technique for photographing individual vortex lines has been developed, the topic will no doubt be the subject of further theoretical and experimental investigations.

Control sites in the lactose operon

from Benjamin Lewin

THE lactose operon has been subjected to such intensive genetic analysis during the past decade that it is perhaps surprising that not all possible classes of mutant were isolated long ago. That the mutants initially taken to identify the promoter in fact correspond to impairment in the action of the cyclic AMP system is, of course, now well known. The wild type lactose operon can be expressed at its full level only in the presence of the cyclic AMP receptor protein (CRP), activated by cyclic AMP. The mutants previously isolated which reduce to 2% of its wild type maximum the expression of the lactose operon all map at the left end of the region between *lacI* and *lacO*, since they fail to recombine with the L1 deletion that covers this region; the reduction to 2% maximum expression is shown also by cells which contain a wild type lactose operon but lack a functional cyclic AMP system. The far left end of the lactose control region therefore seems to provide a DNA site which is necessary for the interaction of CRP and RNA polymerase. Mutants located between this site and the operator have been isolated which allow a high degree of operon expression in the absence of the cyclic AMP system, presumably by creating a site which allows the RNA polymerase

alone to initiate transcription.

The model accepted to explain these results supposes that the promoter of the lactose operon is a complex structure, its left end providing for an interaction with the CRP and its right end providing the site where RNA polymerase binds and initiates transcription. How the interactions at these sites are related is not clear. A prediction of this model is that mutants which reduce the level of expression of the operon by lowering the affinity for RNA polymerase of its binding site should map at the right end of the promoter region.

The isolation of these mutants, constituting the first new mutant class to be reported for some time in the lactose operon, has now been accomplished by Hopkins (*J. molec. Biol.*, **87**, 715-724; 1974). Certain repressor mutants share with promoter mutants the phenotype of reduction in maximum operon expression since they prevent induction, but the promoter mutants alone reduce the basal level of lactose enzymes in the uninduced cell. Using this distinction to identify promoter mutants, Hopkins isolated two classes; the first comprise the previously identified kind of mutation at the left end of the promoter and show failure to respond to the cyclic AMP system; the second class, isolated for the first time, are located at the right end of the promoter region as shown by their ability to recombine with the L1 deletion, and have the properties expected of mutants altered in the interaction with RNA polymerase—they show about 6% of wild type expression in cells with an active cyclic AMP system and this level is reduced to less than 0.5% when the cyclic AMP system is inactive (wild type operons show 2% expression in the absence of the cyclic AMP system). All three mutations of this type lie between the right end of L1 and the operator. The isolation of these mutants opens the possibility of analysis *in vitro* to determine exactly which stage of action of RNA polymerase is inhibited, clearly the next step in identifying the action of this part of the promoter.

The binding of repressor protein to the lactose operon, hitherto thought to implicate only the operator site immediately to the left of the *lacZ* structural gene also may involve more than one site. Using a competitive binding assay for repressor, in which the DNA to be tested is mixed with repressor in the presence of a standard (labelled) operon and the reduction in retention of label on filters is measured, Reznikoff, Winter and Hurley (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2314-2318; 1974) confirm that deletions of the lactose operon have no affinity for repressor and constitutive operator

mutants have a reduced affinity depending on the mutation. Promoter mutants show no reduction in repressor binding, including one deletion which appears to remove virtually all the promoter though leaving the operator intact; the independence of these two sites contrasts strongly, of course, with the overlap between them which is seen in phage lambda. A deletion which removes *lacZ* but does not extend into the operator has the same affinity for repressor as wild type *lac* DNA, confirming that the operator does not extend into the structural gene.

But the strong *lacO^c* mutant DNAs have a much greater affinity for repressor *in vitro* than would be expected from the effect of these mutations in the cell; two mutants which reduce the induction of lactose enzymes in the cell by 100 and 300 times show a reduction in affinity for lactose repressor of only 25 or 30 times. And deletions which remove a large part of the operator show the same affinity for repressor as the operator-constitutive point mutations. This suggests that some common residual binding of repressor might take place at a site present in all these mutant DNAs. When these deletions extend into the first part of the *lacZ* gene, the residual binding remains, but when they extend further, the affinity for repressor is much lowered. This suggests that a secondary binding site is present in the *lacZ* gene, some distance from the operator but within the first thousand bases.

Since the operator seems to be at least 16 bases long, it is very unlikely that this second binding site might be the result of a coincidence of sequence; the probability of independent origin of a sequence with an affinity for repressor as great as 3-4% of that of the operator is low—some *lacO^c* point mutations might well show this order of binding. No obvious role can yet be seen for this second binding site, although it is possible that it might act as a failsafe signal for blocking progress of polymerases that have passed the operator or that it might interact in some secondary structure with the primary operator; there is, however, some evidence from earlier work to argue against both these possible models. The evolutionary origin of this site is an interesting question, some light on which may be cast by sequence studies of the *lacZ* gene as well as the control region. If the second site does play some part in operon function, then its nucleotide sequence must be under two sets of selective constraints, one concerned with its binding of repressor and one with the amino acid sequence specified in β -galactosidase.

articles

Radiation effects in the fusion power programme

Andrew Holmes-Siedle

J. J. Thomson Physical Laboratory, University of Reading, Whiteknights, Reading RG6 2AF, UK

As the prospect of a commercial fusion reactor becomes more real, concerted research is needed into the damage that is likely to be caused by radiation during its lifetime.

IN the past few years, our confidence that we can produce power from controlled thermonuclear fusion has grown, while we have also become more aware of the benefits of fusion power with respect to economy of resources and relative levels of pollution^{1,2}. As a result, the already large international fusion research effort is about to take another step and we are beginning to study the more practical aspects of economics and the detailed technological needs of controlled thermonuclear reactors (CTRs). This new character of the research is prompted by the high measure of confidence among plasma physicists that the necessary levels of plasma temperature, confinement time and density can be achieved so that a net power gain is achieved from fusion reactions. Without fully understanding the plasma behaviour, they believe that, by scaling the plasma confinement system up in size, the conditions for the thermonuclear fusion can be achieved. The choice of reactant will, however, be limited initially to the well known DT system:



Since tritium is not available naturally, it must be bred within the reactor and the only likely reactions available are those of neutrons with lithium.

With these two basic features in mind, some design features of a suitable CTR can already be discerned. Studies of several different forms are under way^{3,4} and preparations are being made to test the feasibility of large scale power production, with the aim of proving the case by the end of the decade⁵. Given a positive answer, then the fusion programme would shift to a phase of developing large, efficient CTRs. Those carrying out design studies are unanimous that we will need to build up a considerable body of new technological skills in order to build such a machine. This article describes the research work which is going on in one particular facet of those skills—radiation effects—and, by outlining the extent of the radiation problems in the CTR, attempts to justify the contention that a lot of research and development remains to be done. Similar treatments could be developed for other areas of CTR technology, a basic list of which is given in Table 1.

Much of the fusion reactor technology can be discussed independently of the form of confinement used, and this is particularly true of the extensive damage and excitation effects which will occur in the confinement system surrounding the reacting plasma and the basic approaches to understanding and dealing with them. We know, for example, that most of the fusion energy released will reside in the neutrons and that, as a result, the materials of the confinement vessel will be severely damaged by these neutrons and accompanying ionising radiation, while the

levels of mechanical stress and temperature will also be high. Radiation and other stress levels have been worked out for some reference designs and I shall draw heavily on the data for two of these, namely the United Kingdom Tokamak design and the Reference Theta-Pinch Reactor (RTPR) designed in the United States⁶.

This arbitrarily chosen pair of reactors represent two divergent approaches to a power-producing toroidal reactor of which the engineering design is quite well developed. Figure 1b shows a cross section of the RTPR and demonstrates the complexity of structure contemplated even in the concept stage. In real life, the simple concentric layers will, of course, be interrupted by ducts and cables leading out from the plasma. The coils for this design are not superconducting. Figure 1a shows a segment of the United Kingdom Tokamak, with some speculative additions by myself to the concept of Mitchell and George⁶. The neutron fluence values noted are not speculative, however, but derive from the neutronics and shielding calculations of McCracken and Blow⁷; however, they are 'worst case' values for a first-wall energy flux value of 10 MW m^{-2} when, in fact, a wall loading value one tenth of this may be adopted. It should be noted that, in both designs, a considerable neutron fluence emerges from the toroid and, if equipment is to be placed within the biological shield, a wide variety of electronic components could receive significant irradiation⁸.

Figure 2 shows calculations of the irradiation rates for the two reactors^{7,9}. Gamma rays are generated by interaction of the neutrons with the structural materials. To the materials scientist, these radiation levels seem unpleasantly large, although not much greater than those experienced near the core of a fission reactor. In the CTR, however, the demands on components and materials are greater in that a high-vacuum chamber, powerful magnets and numerous other complicated systems have to be operated in this environment, as well as the coolant system. The presence of a wider variety of high performance components is likely to compound the problem considerably. Before discussing these specific effects, however, I shall make some general remarks concerning radiation effects at the high levels expected.

Table 1 Major elements of fusion reactor technology

Physics of plasma confinement
Design of breeding blanket
Design of heat transfer system
Design of superconducting magnets (magnetic confinement)
Design of lasers (inertial confinement)
Design of tritium extraction system
Design of fuel injection system
Design of plasma exhaust system
Design for environmental effects (radiation, temperature and so on)

Damage phenomena

The permanent effects of radiation can be divided into two main classes: damage distributed throughout a material ('bulk damage') and damage localised near surfaces ('surface effects'). Under neutron bombardment, three forms of bulk damage are important—atom displacement, gas generation and the generation of solid transmutation products. The degree of bulk damage is still significant in the region outside the breeding blanket, say at a neutron fluence of 10^{18} cm^{-2} . Ductility, creep and stress-rupture/fatigue resistance, as well as the volume of metals, may all be modified and, in some metals, a sharp ductile-to-brittle transition occurs at a fluence of about 10^{20} cm^{-2} at room temperature. Formation of gas bubbles may follow from gas generation and produce changes in mechanical properties by virtue of the accumulation of bubbles at grain boundaries¹. Void formation, a product of very extensive atom displacement¹⁰ produces both swelling and a catastrophic loss of strength. The fluence at which this effect sets in varies considerably with the type of metal, purity and temperature, varying from 5×10^{19} to 10^{22} cm^{-2} . Even at 10^{18} cm^{-2} , before voids appear, the formation of other

microstructures, such as interstitial loops and precipitates of supersaturated constituents, affects mechanical strength.

The resistivity of a metal is increased by the formation of defects, which act to scatter the current carriers. At a fluence of 10^{17} cm^{-2} the resistivity of copper has increased by a few percent and, at 10^{19} cm^{-2} , by a factor of 4 (ref. 11). In superconductors, the critical temperature for superconductivity and the current density are altered slightly by neutron fluences in the 10^{18} cm^{-2} range.

Surface effects will be significant at those free surfaces of metals which are exposed to very high energy fluxes. Neutron energy imparted to the lattice can cause sputtering, and gas bubbles generated near surfaces can produce blisters which may then burst. The first wall is the most heavily bombarded surface and the special surface effects expected here will be described later.

The insulators in a CTR will have a less obvious but still demanding role. Although, in general, metal parts will have been designed for a single role (for example, mechanical support or electrical conduction), insulators often have to fulfil combined mechanical and electrical roles and must therefore be more resistant to change. To take a simple example, a high voltage feed-through insulator has both to support a conductor and to act as a barrier to conduction. This electronic inactivity, however, is not easy to maintain when the material is being heavily excited by nuclear radiation at a high temperature. The wide-band dielectrics are no longer insulators under these conditions (see, for example ref. 12).

To emphasise the general and fundamental nature of these changes it should be pointed out that even at neutron fluence levels of the order of 10^{19} cm^{-2} , crystallinity is destroyed in many inorganic dielectrics at room temperature¹³ and insulators, which are usually transparent in the visible and near ultraviolet, may, at this level of bombardment be opaque because of defect absorptions. Likewise, organic materials, which rely on very specific molecular structures would, under these circumstances, receive sufficient energy to break every chemical bond several times. Thus, unlike a metal, the insulator may undergo a partial breakdown of the main quantum mechanical features which determine its technological use—its low conductivity and wide band gap.

Specific problems

Two well defined functional regions of the toroidal fusion reactor stand out as damage problems—the breeding blanket and the containment magnets. In the first, the energy flux is very large and will include many species other than neutrons (α particles, γ rays, soft X rays, energetic ions, thermal plasma and so on); in the second, a critical level of performance has to be maintained. Further, throughout the reactor, at points yet to be determined, there will be electrical and electromechanical components which must operate reliably, such as valves, relays and cables. I shall deal with the main functional regions first.

The first wall will be a thin metal barrier between the reactor vacuum and molten lithium coolant. The most commonly quoted metal for the wall is a niobium alloy but other refractory metals (V, Mo) and several other groups of metals are being considered, for example austenitic stainless steels, nickel alloys, sintered aluminium powder, silicon carbide and graphite. There have been no tests on these metals with 14 MeV neutrons at high temperatures and fluences because of the difficulty of generating intense neutron beams of this energy, but irradiations in high flux reactors (neutron energies in the range 0.1–5 MeV have been carried out on many stainless steels and the information has been supplemented by gas implantation experiments¹⁰. Major stresses on the first wall will, however, be the repeated thermal shocks as the reaction is started and stopped cyclically, and the high stresses created by the

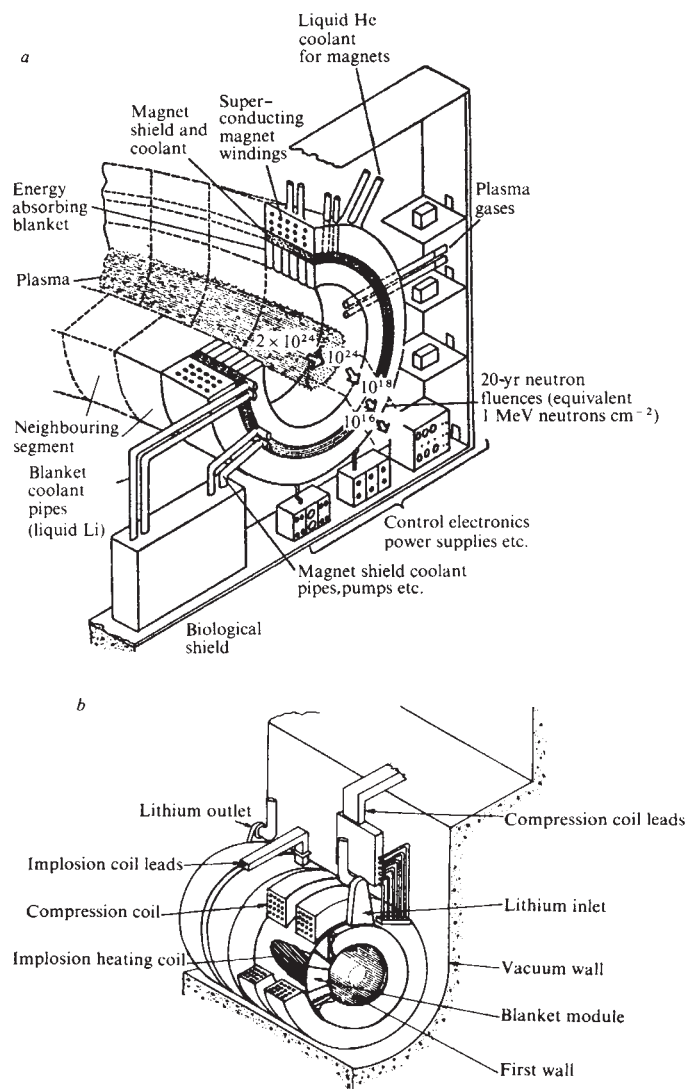


Fig. 1a. Segment of the United Kingdom Tokamak fusion reactor showing calculated neutron fluences for a life of 20 yr. Structural design by Mitchell and George⁶. External equipment shown may have to be placed outside the biological shield. Outside diameter of segment is 4 m. **b.** Cross section of the Reference Theta-Pinch Reactor (RTPR) showing functional regions of the toroid⁹. Outside diameter of segment is 1.5 m.

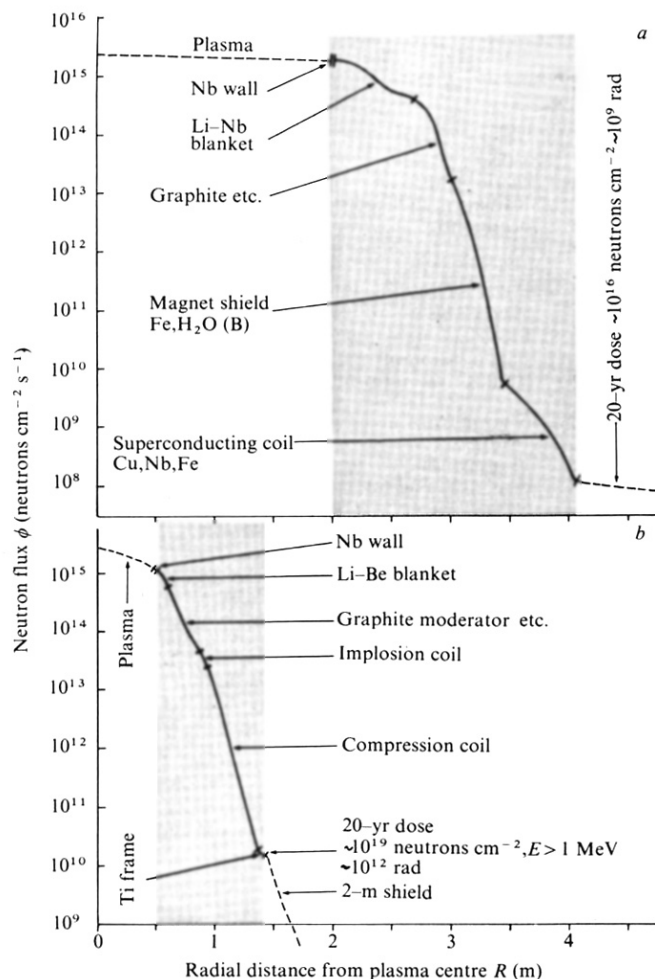


Fig. 2 Radiation levels in (a) Tokamak⁷ (10 MW cm⁻²) and (b) RTPR⁵ (6.7 MW cm⁻²) showing larger size and heavier shielding in Tokamak. Shading indicates functional structures.

magnetic confinement field. Such a cyclical mode of operation will vary in severity between the theta pinch and "DC" type of reactor but, in both cases, cyclical stresses will be much greater than have been experienced in fission power reactors, which are operated in a 'steady state'. Unfortunately, theories of fatigue failure are not well advanced and thus it is fair to say that we do not yet know whether first wall can be fabricated which will take the stresses required at an economic size. Mitchell and Booth, however, have evolved a flexible design of helium-cooled cell for the first wall which will allow the designer to minimise the mechanical forces acting on the wall¹⁴.

The main surface effects which have to be considered for the first wall are:

- thermal evaporation caused by the high energy flux;
- sputtering by the plasma ions, which will include deuterium, tritium, helium and some heavier ions originating in the wall;
- blistering arising from gas implantation;
- neutron sputtering.

These effects are doubly deleterious to reactor operation since they result both in erosion and the introduction of impurities into the plasma. The flux, ϕ , of ions from the plasma can be estimated using the expression

$$\phi = [5.6 \times 10^{13} W/F] \text{ cm}^{-2} \text{ s}^{-1}$$

where W is the wall loading in MW m⁻² and F is the fraction of ions which undergo fusion. Sputtering yields are only known to within a factor of 2 to 3 for light ions¹⁵ and the erosion rates of neutron sputtering and blistering effects

are not yet established, since the phenomena have only recently been discovered. Blistering effects vary with ion energy, target temperature and dose rate¹⁶; apart from the spalling of the blistered surface, the blisters also act as thermal impedance and should lead to enhanced surface evaporation of the first wall. Neutron sputtering was thought to be negligible¹⁵ but Kaminsky and coworkers¹⁷ have recently found rates to be higher than for light ions (as high as 0.25 atoms per neutron), which could lead to intolerable erosion rates.

A major problem in the design of the theta-pinch type of reactor is that the first wall must be coated with an insulator to withstand the transient electrical field generated when the plasma is made to contract or implode⁴. Operating at a temperature of over 600° C, it must withstand a field of about 10⁷ V m⁻¹ for about 1 μs. When the plasma ignites, the insulator will be exposed to a thermal shock and a burst of particles. Although Bunch and coworkers⁸ feel that some forms of alumina ceramic may have the intrinsic mechanical strength to withstand the thermal effects, it is not yet known whether such a material will maintain its dielectric strength or integrity under the multiple particle and photon bombardment effects which will accumulate. Table 2 lists some of these effects. It is worth noting that the peak ionising dose rate in this insulator (A. G. H. S., unpublished) is likely to be greater than a rate which could lead to the transport of amperes of current per square metre of surface, and the material cannot be considered as insulating at all times.

Superconducting magnets face both a radiation-heating problem and a radiation-damage problem. The energy flux of neutrons at the magnet can cause excessive heating unless shielding is added within the confinement toroid. The Tokamak design incorporates such a secondary shield of water-cooled steel. McCracken and Blow⁷ have made a detailed study of the neutron-induced changes in aluminium and copper, two of the materials which may be used as conducting matrices for superconductive filaments; these authors conclude that the neutron fluence over 20 yr can be allowed to rise to 10¹⁸ cm⁻² if the magnets are allowed occasional annealing treatments, at room temperature or above. Copper has advantages in strength over aluminium, and neutron irradiation actually improves the yield stress levels in copper, although more creep than usual occurs under neutron irradiation because of the enhanced atom motion. Since the mechanical forces on conductors and the structure of powerful magnets are very high, creep effects may be significant. Conductivity degradation is also one of the more difficult effects to simulate—mechanical forces have to be applied to metal and dielectric samples at high neutron fluxes and extremes of temperature. At the moment test sources with the required neutron fluxes (about 10¹⁵ cm⁻² s⁻¹) are not yet available and research may have to be started using fission neutrons and ion beams which can give the correct knock-on energies although only over limited volumes of material.

In the theta-pinch design, the compression coils are not superconducting and no special measures are taken to reduce neutron heating. Neutron fluxes will be more than four orders of magnitude higher but some of the damage will anneal as it is formed.

The insulation of the magnet coil conductors presents more of a problem in both designs. At present, thin organic layers are placed between each conductor, with organic potting to give the very necessary binding support. The total radiation doses at the coil are probably high enough to destroy the mechanical properties of most organic insulators, and inorganic insulators will probably have to be used. In the latter materials, displacement and transmutation can permanently increase conductivity and reduce breakdown strength; internal buildup of space charge can lead to very high internal fields and spontaneous breakdown

Table 2 Fusion reactor technology—summary of possible radiation effects problems in insulators

Physical phenomenon	Major functional effect	Class of components strongly affected	Basic studies (if any) needed	Possible technological solutions
(1) Electrical effects Generation of displacement defects leading to: —Changes in carrier mobility* —Generation of defect levels Amorphisation leading to: —Loss of directional properties —Band-tailing and so on Transmutation, precipitation of products Space-charge build-up Photoconduction Changes in secondary emission and photoemission	Changes in conductivity Changes in dielectric loss Changes in dielectric constant Piezoelectric device failure Changes in dielectric breakdown strength at precipitates and pores Internal breakdown Unwanted potentials Transient conductivity Changes in first-wall emission efficiency	First wall Standoff insulators Capacitors Oscillators Acoustic devices All insulators	Effects of defects on carrier transport Radiation induced defect production Physics of amorphous solids and amorphisation Electrical breakdown Precipitation of transmutation products Charge traps in insulators Photoconductivity in insulators and interfaces Surface barriers in solids	Development of special neutron-tolerant glasses or ceramics with special doping grain size, and microstructure Development of methods for depositing neutron-tolerant films of insulators Insulators of optimised conductivity under irradiation (controlled leakage)
(2) Optical effects Defect production Surface loss of stoichiometry	Changes in (a) transmission and reflectance (b) absorptivity/emissivity relations controlling equilibrium with plasma	First wall Windows Light guides	Colour centres Emissivity Surface radiation damage Surface reactivity	Passivated, non-darkening surface coatings Radiation-tolerant light guides
(3) Thermal effects Colour-centre production Lattice disruption and impurity precipitation	Decrease in radiative heat transfer Decrease in heat transfer by way of lattice	First wall Lagging of coolant pipes Superconductor insulation	Colour centres Lattice vibrational modes	Dielectrics of high thermal conductivity
(4) Mechanical effects Thermal shock Radiation-induced swelling (anisotropic) from defect formation Enhanced atom motion Phase changes and so on Sputtering	Fatigue fracture Microcracking Debonding Loss of strength Creep Increase of friction Loss of directional properties and strength Erosion of thin layers	All dielectrics, especially first-wall coating, spacers and coil insulators Precision parts Valves Piezoelectric devices First-wall coatings	Microstructure and strength of materials Generation and rates of annealing of point defects and voids Atomic diffusion under irradiation Friction and micro-structure Radiation-induced phase changes Sputtering physics	Use of isotropically expanding dielectrics (e.g. glasses), Use of creep to relieve swelling Optimisation of powder size in ceramics Insulators with (a) Rapidly annealing defects (b) low void formation Special solid lubricants
(5) Chemical effects Reduction by hot hydrogen Diffusion of, for example, oxygen from insulators	Reduction of oxides, nitrides etc. Deoxygenation and so on of oxide insulators by Li, Nb and so on	First wall Other coatings First wall Oxide/Nb/Li system	Reactions of atomic hydrogen at surfaces Oxygen diffusion in metals and insulators	Passivated coatings Oxygen diffusion barriers

* Carrier mobility has a strong influence on radiation induced conductivity in insulators.

effects. Finally, the leakage currents arising from photoconductive effects cannot be ignored. In the case of the theta-pinch coil design, the coil insulators may experience dose rates in the region of 10^6 rad s^{-1} and photoconductivity may cause the resistivity of inorganic insulators to change from a normal 10^{18} to 10^{10} ohm cm during the pulse, leading to leakages in the ampere range. Also, the use of implosion heating requires the application of a fast pulse at a voltage of more than 100 kV at the coil terminals; local fields could thus exceed the dielectric breakdown strength in the irradiated insulators. In the case of superconducting coils, fields are low in normal circumstances but if the superconducting-to-normal transition occurs, a voltage of up to 20 kV can appear across the terminals. Doses in the well shielded Tokamak magnets, however, will probably be only $\sim 10^2 \text{ rad s}^{-1}$.

Proceeding from the plasma outwards, the first region

in which electronic devices might be placed would be in the void between the coils and the biological shield. Some control circuits and actuators for the coolant and vacuum pumping systems may need to be near the torus. Radiation dose rates can be reduced by local shielding but a 20-yr zero-shield figure of $10^{16} \text{ neutrons cm}^{-2}$ and 10^8 rad for this void region is not unreasonable to expect. This is a level which requires a special selection of all semiconductor and dielectric parts (including organics) used in this region*.

There remains a class of damage problems which cannot yet be described in detail but of which the outlines are clear. The nuclear fuels, deuterium and tritium, must be passed into the reactor, probably by means of particle (pellet or ion) accelerators; currents must be passed through the plasma; impurities must be "diverted"¹¹; spent fuel must be pumped out; eddy currents must be suppressed. Each of these functions requires the presence of a virtually

independent subsystem—as yet of uncertain design—with elements near to the plasma. The materials needed and the fluxes to which they will be exposed will become known as the detailed geometry of reactors is developed (see, for example ref. 19) but it is not too early to develop a general knowledge of the effects of high fluxes of neutron on those types of metal which may be used and of combined neutron- γ effects on the dielectrics. Some idea of the materials of interest can be gained by examining the materials commonly used in various component technologies (see, for example refs 8 and 20). A case in point is the window problem in the laser fusion reactor (see for example, paper 8 of ref. 4). Light transmission, reflectivity and dimensional stability are all key performance parameters in this precision optical system and, as Table 2 shows, all are affected strongly by high levels of neutron and γ irradiation (estimated doses are 2×10^{20} cm $^{-2}$ yr $^{-1}$ and 10^{11} rad yr $^{-1}$, respectively).

Basic research needed

This list of hazards for the CTR system need not make us drop the idea of building such a machine. The research on radiation effects done in support of the fission reactor programme and the 'hardening' of semiconductor devices for aerospace use has shown that solutions can usually be found for each problem if an understanding of the phenomenon is first achieved; however, such solutions have often in the past proved to be highly individual and specific (see, for example, the beneficial use of lithium in silicon solar cells²¹ which, however, does not benefit silicon transistors) and take a long time to put into effect. Thus, if fusion technology is to proceed according to a proper timetable, a form of 'preventive research' must be undertaken in which the basic phenomena of radiation effects for any material of interest are studied to the point that any potentially crippling problems of degradation will have been noticed before the development of that material has proceeded unduly far²⁰.

For metals, some of the damage phenomena described in the earlier sections are already being studied as part of fast fission reactor development, but these must be widened to include surface effects and to embrace a wider range of metals. For dielectrics, Table 2 provides a comprehensive list of damage phenomena. In looking for neutron-tolerant dielectrics, the glasses are of special interest because they are not prone to the anisotropic expansion which can lead to loss of strength in crystalline insulators²². The fission reactor programme has already demonstrated the tolerance of certain refractory oxides and other ceramics to radiation. It is assumed that semiconductors will be virtually excluded from the high flux regions⁸.

In the field of theory, recommendations have been made for development in theoretical models for creep, embrittlement, crack formation and void formation, especially in the refractory metals²³; for dielectrics, models will be required for damage to electrical and optical properties as well as to mechanical properties. Up to now, the electrical and optical properties of insulators have not been of central importance in the nuclear energy field and as a result, there is a smaller body of damage theory on which to base future work than for metals. Furthermore, the technologically important oxides (such as silica, alumina and zirconia) are crystallographically complex and for this reason have not received the required degree of attention from theoreticians as media for band theory and defect studies.

Aims and timetable

Having stated the topics for basic research, I shall briefly lay out a set of technological aims for dealing with radiation effects in CTR components, based on a timetable for general CTR development by Hancox²⁴, as follows:

Metals. (1) Development of structural metals with minimum change in ductility, creep, stress rupture, fatigue and swelling under high neutron fluences at high temperatures (and with high resistance to corrosion by the breeding material). (2) Development of conductor/superconductor with the minimum change in resistivity and creep strength at very low temperatures and moderate neutron fluences. (3) Control of surface erosion phenomena such as blistering, sputtering and gas desorption.

Insulators. (1) Development of structural ceramics and glasses and a range of electronically passive thin coatings resistant to radiation-induced swelling, cracking, leakage, high voltage breakdown and chemical attack. (2) Development of special deposition methods for insulating coatings. (3) Development of radiation tolerant light-handling media. (4) Development of dielectric materials to perform special electronic functions under high neutron- γ ionisation.

Taking 1979–84 as the period during which feasibility of fusion reactors will be tested, we can also draw up a tentative timetable giving a concerted fundamental and technological development and which will permit the successful construction of short lived experimental reactors by 1979 and long lived, economic prototype reactors in the early 1980s, as follows:

1974–79: Preparatory research. Fundamental research on damage and structure of reactor materials, especially dimensional stability of metals and electronic stability of dielectrics; initial development of specific radiation-tolerant structures (especially electro-mechanical or electro-optical devices) required for the demonstration power reactor to be designed in the 1980–82 period; Simulation of neutron- γ , ultraviolet and plasma effects in metals and insulators using electron, ion and proton bombardment and the highest fluxes of non-fusion neutrons available; experiments on lower levels of neutron fluxes from early fusion plasma experiments (as available) for comparison with non-fusion sources.

1979–82: Feasibility testing. Fabrication of prototype radiation-tolerant materials and devices for a demonstration reactor; exposure of samples in feasibility-test reactor; full design of test rigs for demonstration reactor.

1982–90: Demonstration reactor. Fabrication of radiation-tolerant materials and devices for demonstration reactor; accelerated life tests for 20-yr operation of prototype reactor; development of second generation of radiation tolerant materials and devices for a possible prototype commercial CTR.

Conclusions

This review of one branch of fusion reactor technology has been made with the idea of distinguishing problems in radiation effects which deserve to be investigated during the next few years, even before reactor designs are finalised. The field of insulator study has perhaps been given greater emphasis than that of metals because the latter has been dealt with at length in recent conferences and workshops while the latter has been somewhat neglected by comparison; thus, the multiplicity of effects produced in materials with a wide band gap has been stressed. It seems, however, that the strength of metals under irradiation may be the limiting factor in the life and efficiency of controlled thermonuclear reactors and thus an extensive programme of research and testing of metals should continue for the rest of this decade. For both insulators and metals, techniques of predicting and dealing with radiation effects will need to be improved and 'preventive research' carried out on many of the less obvious materials. The intelligent direction of research may well help to achieve the target of useful fusion power in the national grid not long after the beginning of the next century.

I acknowledge the liberal assistance of Dr G. McCracken in supplying details and comment on the United Kingdom Tokamak, making constructive comments on the manuscript and allowing the use of some unpublished text material. F. W. Clinard, jun., of Los Alamos Scientific Laboratory made some useful comments on Table 2 in proof in a recent discussion.

Note added in proof: For Tokamak reactors it may be required that the torus as a whole must not constitute a closed conducting loop. A completely insulating segment must thus be inserted in the torus, the inner edge of which will be exposed to the plasma. This segment or "spacer" will have to maintain strength, dimensional stability and electrical properties over the range of radiation doses shown in Fig. 2a.

¹ Marshall, W., *Nature*, **243**, 384–388 (1973).

² Hirsch, R. L. *et al.*, *Fusion Power, An Assessment of Ultimate Potential*, WASH-1239 (US Atomic Energy Commission, February 1973).

³ Pease, R. S., *Nature*, **248**, 713 (1974).

⁴ IAEA Workshop on Fusion Reactor Design Problems, January 1974, Culham, Papers 1–21; *Nuclear Fusion* (to be published).

⁵ Ribe, F. L., Dudziak, D. J., *et al.*, *LASL Controlled Thermo-nuclear Research Program*, Report No. LA-5250-PR (Los Alamos Scientific Laboratory, New Mexico, June 1973).

⁶ Mitchell, J. T. D., and George, M. W., *A Design Concept for a Fusion Reactor Blanket and Magnet Shield Structure*, Report CLM-R121 (UKAEA Culham Laboratory, Abingdon, 1972).

⁷ McCracken, G. M., and Blow, S., *The Shielding of Superconducting Magnets in a Fusion Reactor*, Report CLM-R120 (UKAEA Culham Laboratory, Abingdon, 1972).

⁸ Holmes-Siedle, A. G., *Rep. Prog. Phys.*, **37** (in the press).

⁹ Bunch, J. M., Clinard, F. W., Dudziak, D. J., Green, W. V., and Krakowski, R. A., *An Evaluation of Major Material Problems Anticipated for the Reference Theta-Pinch Reactor (RTPR)*, Report No. LA-UR-73-1653 (Los Alamos Scientific Laboratory, New Mexico, 1973).

¹⁰ *Proc. BNES European Conf. on Voids Formed by Irradiation of Reactor Materials* (edit. by Pugh, S. F., Loretto, M. H., and Norris, D. I. R.), Reading, March 24–25, 1971).

¹¹ Burger, G., Meissner, H., and Schilling, W., *Phys. Stat. Sol.*, **4**, 281 (1964).

¹² Hughes, R. C., *Phys. Rev. Lett.*, **30**, 1333–1336 (1973).

¹³ Primak, W., and Kampwirth, R., *J. appl. Phys.*, **40**, 2565 (1969).

¹⁴ Mitchell, J. T. D., and Booth, J. A., *Wall Loading Limitations in a Helium Cooled Fusion Reactor Blanket*, UKAEA Report No. CLM-R 126 (1973).

¹⁵ Behrisch, A., *Nuclear Fusion*, **12**, 695 (1972).

¹⁶ Erents, S. K., and McCracken, G. M., *Radiat. Effects*, **18**, 191 (1973).

¹⁷ Kaminsky, M., Peavy, J. H., and Das, S. K., *Phys. Rev. Lett.*, **32**, 599 (1974).

¹⁸ Kulczinski, G. L., *Major Technological Problems for Fusion Power Stations*, University of Wisconsin Report No. FDM 33 (November 1972).

¹⁹ Fraas, A. P., *Conceptual Design of the Blanket and Shield Region and Related Systems for a Full-scale Toroidal Fusion Reactor*, Report ORNL-TM-3096, (Oak Ridge National Laboratory, Tennessee, May 1973).

²⁰ Holmes-Siedle, A. G., *Proc. IEEE* (in the press).

²¹ Faith, T. J., Brucker, G. J., and Holmes-Siedle, A. G., *IEEE Trans. nucl. Sci.*, **NS-15**, (6), 61 (December 1968).

²² Wilks, R. S., *Radiation Effects in BeO, Al₂O₃ and MgO*, UKAEA Report AERE-R5596 (1967).

²³ Report of Working Group 6, IAEA Workshop on Fusion Reactor Design Problems, January 1974, Culham; *Nucl. Fusion* (in the press).

²⁴ Hancox, R., *Fusion Reactor Studies in the United Kingdom*, Report CLM-P329, (UKAEA Culham Laboratory, Abingdon, 1972).

Evidence for the breakup of eastern Gondwanaland by the early Cretaceous

Rudi G. Markl

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York 10964

An analysis of early Cretaceous marine magnetic anomalies of south-western Australia helps to piece together eastern Gondwanaland. It is suggested that India once abutted Western Australia.

THE seafloor spreading history of the portion of the Indian Ocean basin between the Ninetyeast Ridge and Australia, the Wharton Basin, is poorly understood. Most Gondwanaland reconstructions have placed the eastern coast of peninsular India against Antarctica, generally leaving a gap of about 15° of longitude between India and Australia^{1–5}. Some workers have placed the eastern coast of India against Western Australia^{6–8} and yet others have favoured placing Indonesia, south-east Asia, even China, against Western Australia^{9–11}. I present here new evidence pertaining to the reconstruction of eastern Gondwanaland; the recognition of early Cretaceous marine magnetic anomalies adjacent to south-western Australia.

Anomalies M-1 to M-11 inclusive of Larson and Pitman¹² have been identified between the northern edge of the Naturaliste Plateau and the Australian mainland. The lineations, referred to here as the 'Perth sequence', vary in strike from 040° near the plateau to 020° near the mainland; they demonstrate that the age of the oceanic crust increases toward the continent (Fig. 1). Although somewhat older crust may exist beneath the continental

margin, the presence of anomaly M-11 dates the separation of India (?) and Australia in this region at 127 Myr BP, or earlier. This date is nearly coincident with that determined for the opening of the South Atlantic Ocean¹³.

Larson *et al.*¹⁴, suggested that the time scale of Larson and Pitman¹² should be compressed by 10–15% relative to anomaly M-22. A 10% compression has been used here.

Anomalies M-2 and M-4, the most distinctive of the younger half of the M series, correlate well from track to track (Fig. 2). The correlation of anomaly M-2 is dashed south of profile EL48 because tracks 1106-2 and EL54 cross onto the Naturaliste Plateau in this vicinity. The model anomaly profile (Fig. 2) uses a magnetic layer 500 m thick, with a remanent magnetisation intensity of 0.012 e.m.u. cm⁻³. Models were also calculated for remanent magnetisation acquired at palaeolatitudes 50–70° S and for subsequent rotations of the plate of as much as 40°, both clockwise and anticlockwise. The correspondence to the observed data, however, was not greatly affected. The model profile used here (Fig. 2) is for latitude 60° S and no subsequent rotation. The correspondence between the observed anomalies and this model is comparable to that of the equivalent portions of the Cape basin sequence¹⁵ and the Keathley sequence¹⁵, which also exhibit relatively slow spreading rates.

The identification of the M series is substantiated by Site 259 of the Deep Sea Drilling Project (DSDP), which gave

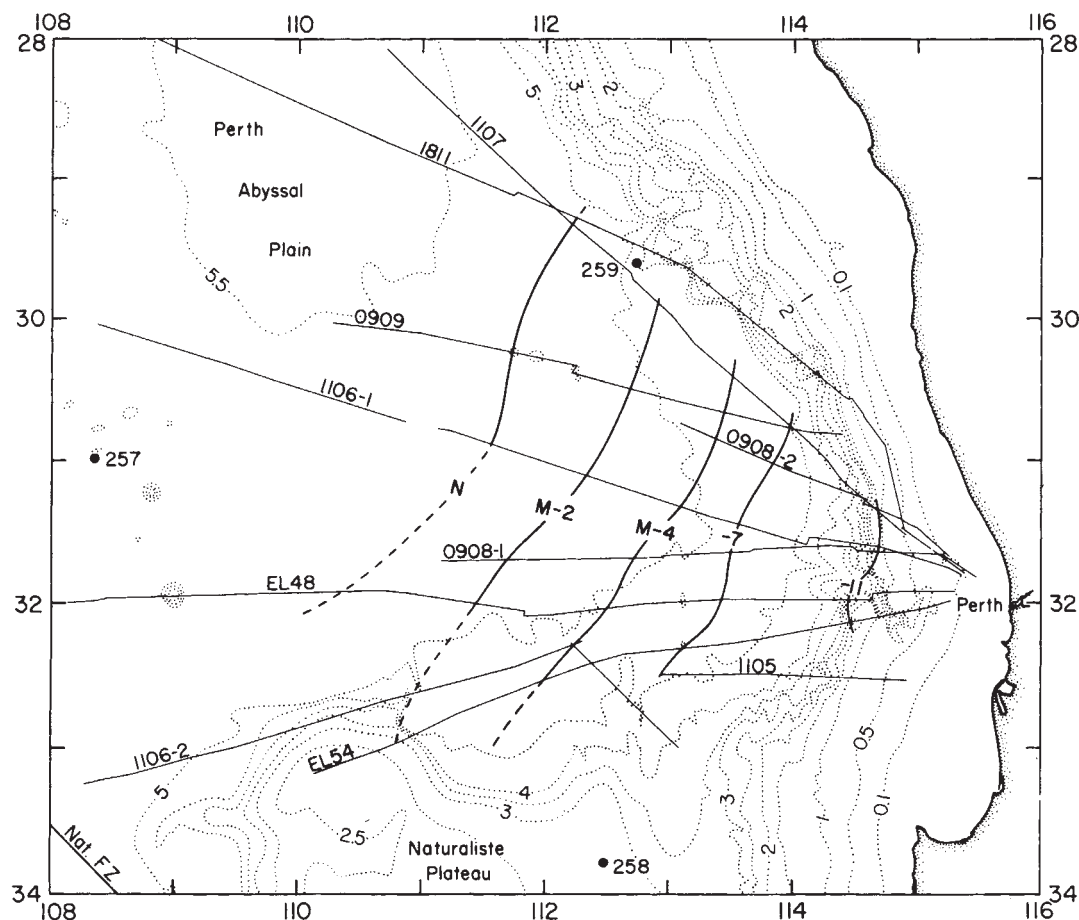


Fig. 1 The Perth sequence of Mesozoic (M) magnetic lineations showing the portions of the ship tracks corresponding to the observed profiles shown in Fig. 2. ●, DSDP site numbers; N, negative magnetic anomaly. The bathymetric contours are in corrected metres.

a basal sediment age of greater than 110 Myr (ref. 16). Calculations based on anomalies M-2 (116 Myr BP) and M-11 (127 Myr BP) indicate that the (half) spreading rate during this period was 2.3 cm yr^{-1} . Site 257, located in the late Cretaceous 'quiet zone'¹⁷, yielded an age from near basal sediments of 102 Myr (ref. 18), which corresponds to a (half) spreading rate of 2.1 cm yr^{-1} between Site 257 and the M-2 lineation.

A negative anomaly just west of (younger than) the Perth sequence has also been correlated (Figs 1 and 2). This anomaly is probably equivalent to a negative anomaly adjacent to the Cape basin sequence which may represent a magnetic reversal¹³.

Sclater and Fisher¹¹ identified east-west trending lineations (Fig. 3) which indicate that the age of the crust of the northern Wharton Basin increases towards the south. The assumption that this spreading regime also created the crust of the southern Wharton Basin led them to conclude that India could not have abutted Australia in Gondwanaland.

The Perth sequence, although oriented at about 55° to the younger lineations of Sclater and Fisher¹¹, was initially considered to be related probably to some segment of their anomaly pattern, perhaps to the sliver bounded by their proposed 96° E and Investigator fracture zones (Fig. 3). The intervening 20° of latitude between anomaly 33b (ref. 11) and anomaly M-1 would represent the late Cretaceous (115–85 Myr BP) magnetic quiet period. This scheme for the origin of the Perth sequence would require an average (half) spreading rate of more than 7 cm yr^{-1} during the quiet period, and a shift of the pole of rotation commensurate with the required change from NW–SE to N–S spreading. The (half) spreading rate between DSDP Site 212 (approximately 95 Myr BP) (ref. 19), which is 330 km south of anomaly 33b (Fig. 3), and Site 256 (basal sediment age 101 Myr) (ref. 18), is about 8 cm yr^{-1} . However, the fact

that Site 257 is fully 1,100 km south-east of Site 256, has the same age as Site 256, is obviously inconsistent with this scheme. The slow spreading rate of the Perth sequence (2.3 cm yr^{-1}) also implies that the achievement of a 7 cm yr^{-1} average spreading rate between anomalies M-1 and 33b is not realistic. Additional magnetic evidence from the southern Wharton Basin offers an alternative mode of origin for the Perth sequence.

The overall 'unquietness' of the supposed quiet zone in the southern Wharton Basin is disturbing. Peak-to-peak amplitudes of up to 700γ are common. These anomalies seem to be unrelated to the basement morphology and in many instances they can be correlated from track to track. The similar basement ages of DSDP Sites 256 and 257, combined with a varying degree of axial symmetry on magnetic profiles in the quiet zone between these sites, led to the recognition of anomalies believed to be M-1 to M-22 inclusive, between the northern extension of Broken Ridge (Fig. 3) and Ninetyeast Ridge. Anomalies M-2 and M-4 can be traced for more than 400 km, striking 040° (Fig. 3). The identification of the older anomalies is, however, based upon only one track—Vema 18—(Fig. 4). In Fig. 4 this profile is shown with a model anomaly profile for remanent magnetisation acquired at 60° S and 0° declination. The character of anomalies M-1 to M-11 inclusive and their correlation with the model, is similar to that of the anomalies of the Perth sequence; the correlation of the observed and model profiles between anomalies M-11 and M-22 is poor. The spreading rate between anomalies M-2 and M-22 is 1.6 cm yr^{-1} . A negative anomaly just east of anomaly M-2 has been correlated between tracks, but it is questionable whether this anomaly is equivalent to the negative anomaly west of the Perth sequence. Site 255 lies 70 km south-east of the extrapolated trend of anomaly M-2. It is, however, located on top of Broken Ridge, and it terminates 200 m above the basement, in 80-Myr-old material¹⁸. Therefore it

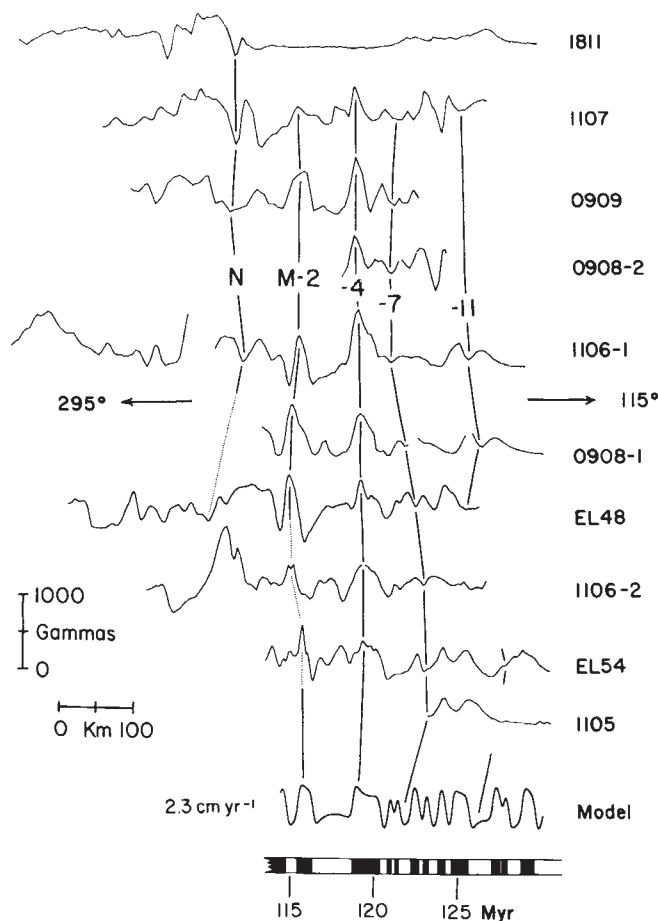


Fig. 2 Observed magnetic profiles of the Perth sequence projected perpendicular to the strike of the lineations and plotted above the model anomaly profile; the latter was computed from the magnetic block model of Larson and Pitman¹².

probably has no potential as a support for the identification of the Broken Ridge sequence. Lamont-Doherty has no data north of the Vema 18 track but profiles Lusiad-3 Project Magnet 541 (refs 11, 20) show anomalies which may represent M-2 and M-4. Along the LU-3 track the possible M-2 anomaly lies 155 km north-west of Site 256 (101 Myr BP), which would correspond to a (half) spreading rate of only 1.0 cm yr⁻¹. The LU-3 data suggest that the lineations may curve toward the north (Fig. 3). If the Project Magnet 541 data are also used, a 75 km sinistral offset of the lineations just north of the Vema 18 track is implied. An offset here is not altogether unlikely, in view of the trend of a previously unreported fracture referred to here as the 'Naturaliste fracture zone' (Fig. 3). The strike of this 650 km long fracture zone is well defined by seismic and bathymetric data in the vicinity of the Naturaliste Plateau.

The axis of the spreading centre inferred to have generated the Perth and Broken Ridge sequences is not well delineated. Figure 5 shows observed magnetic profiles from the quiet zone between the Perth and Broken Ridge sequences. The corresponding track lines, as well as the trends of correlated anomalies, are shown in Fig. 3. The magnetic trends consistently strike NE-SW to NNE-SSW, providing another argument against the probability of a direct relationship between the Sclater and Fisher pattern and the Perth sequence. A prominent negative anomaly defines the axis of symmetry on profiles EL45 and 1105 (Fig. 5). With the exception of the central anomaly, the large posi-

tive anomalies between C and C' on profile 0909 are associated with large seamounts. North of profile 0909 the position of the axis is based upon the overall symmetry of the magnetic profiles. Bathymetric and seismic reflection data show that the northern half of the inferred axis of spreading coincides with the axis of a regional topographic and basement high. The basement topography across the axis is generally smooth compared with active ridge crests although discrete seamounts are abundant in the axial zone between latitudes 28° and 29° S. The positions of basement scarps which flank the axis are shown in Fig. 3. The average thickness of sediment in the axial zone varies from 700 m at the southern end to 300 m in the north.

Extrapolation of the spreading rates of the Perth and Broken Ridge sequences indicates that the inferred spreading centre would have become inactive by about 77 Myr BP. The average basement depth in the axial zone is 5,275 m. This depth compares favourably with the depth predicted (approximately 5,400 m) for a slow spreading ridge 77 Myr after the cessation of spreading²¹.

There is a reasonable case for the existence of an extinct spreading axis in this region. An approximate symmetry exists in the magnetic profiles along several tracks. The axis of symmetry is essentially perpendicular to the trend of the Naturaliste fracture zone and parallel to those of the Perth and Broken Ridge sequence lineations. The distance from the axis to these sequences is roughly proportional to their respective spreading rates, and it lies essentially midway between two holes of the same age (Sites 256 and 257).

Although the proposed late Jurassic-late Cretaceous spreading centre provides an explanation for the problem of the widely-spaced but similarly aged DSDP sites in the southern Wharton Basin^{18,22}, it bears problems of its own. Anomaly M-11 lies adjacent to Australia and is the oldest anomaly of the Perth sequence. The oldest identifiable anomaly of the poorly documented Broken Ridge sequence is, however, M-22, and there is room between M-22 and the Ninetyeast Ridge for still older crust. This apparent discrepancy in the time of inception of spreading (as well as the apparent asymmetry of the spreading rates of the two sequences) may be alleviated by the fact that when the M-2 lineation (from Vema 18 southward) of the Broken Ridge sequence is rotated into alignment with the M-2 lineation of the Perth sequence, the former sequence is not juxtaposed with the latter. Instead, the Broken Ridge sequence lies to the south-west, on the opposite side of the Naturaliste fracture zone. This rotation of 13.4° about a pole at 40°N; 175°E leaves the Naturaliste fracture zone perpendicular to the lineations.

Another difficulty which must be explained is the presence of the northern extension of Broken Ridge, which roughly parallels the magnetic lineations (Fig. 3). Seismic profiles show that the northern end of this feature (delineated by the 3,000 m isobath) resembles the Naturaliste Plateau. Although it is possible to fit these two plateaux together, the presence of the northern extension on crust which post-dates the inception of spreading (anomaly M-11) by 15–20 Myr precludes a simple rifted origin.

As already stated, the spreading centre in the southern Wharton Basin might have become inactive by 77 Myr BP. Anomaly 33 is not discernible in the axial zone, and as the magnetically quiet period is believed to have been marked by a pulse of rapid spreading¹², the cessation of spreading probably occurred earlier. The presence of anomaly 33b in the northern Wharton Basin¹¹ indicates that the spreading centre which generated it was active by about 80 Myr BP. No structural feature is known in the area between the two regimes (lat. 20°–22°S) which could suggest the location of a possible boundary between them. The magnetic anomalies in this area are typical of a quiet zone. One way to reconcile these two spreading regimes is to postulate that the older spreading axis jumped to an east-west

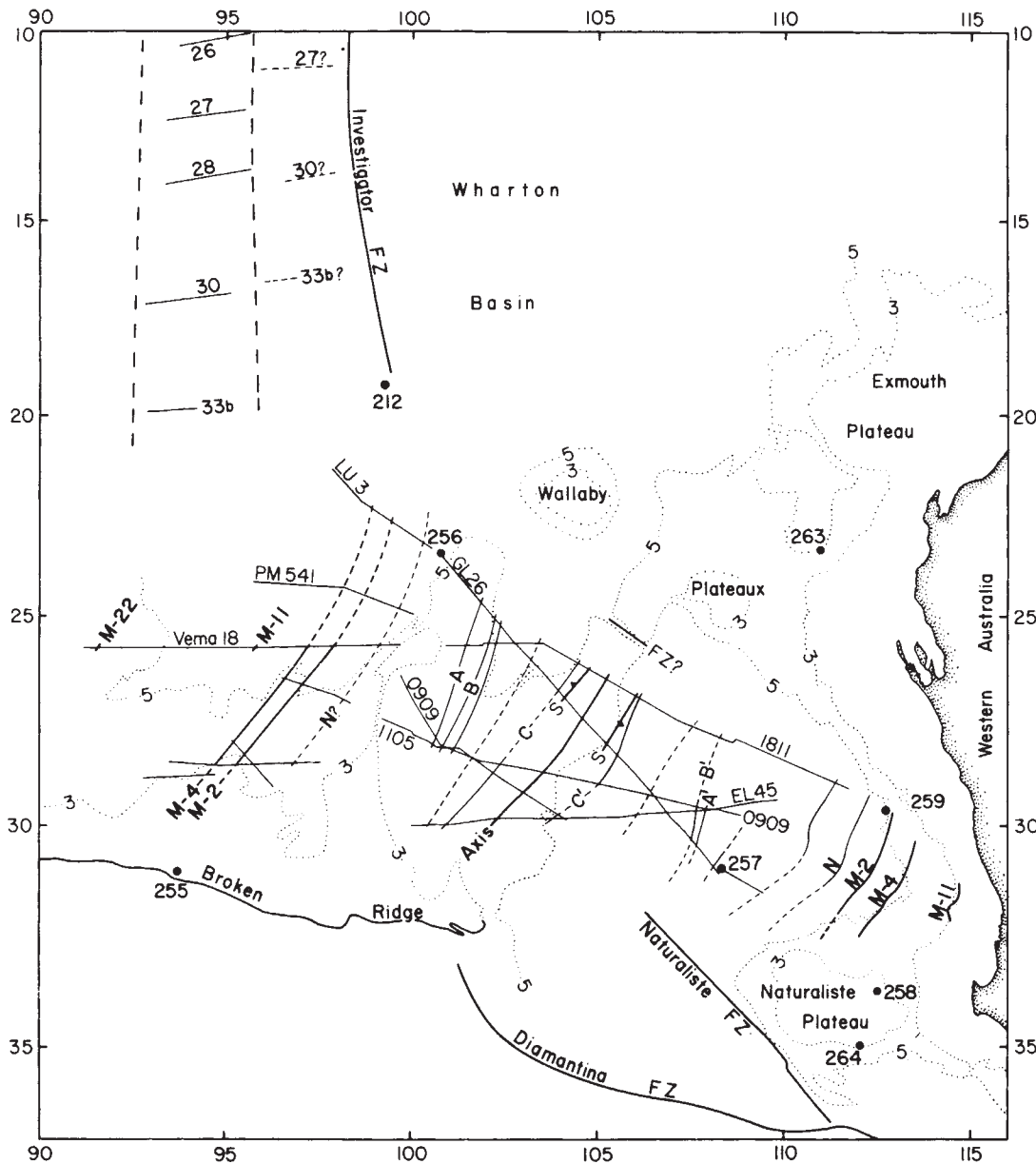


Fig. 3 Map of the Wharton basin showing the southern half of the anomaly pattern of Sclater and Fisher¹¹ (anomalies 26–33b), the Perth and Broken Ridge sequences of Mesozoic (M) magnetic anomalies, and the axis of the proposed spreading centre. Magnetic lineations A, A', and so on, are derived from observed profiles shown in Fig. 5. Wall. Plat., Wallaby Plateau; N, correlated negative magnetic anomaly; dotted lines labelled '3' and '5', the 3,000 and 5,000 corrected meter isobaths respectively; S, basement scarps; unlabelled lines crossing the Broken Ridge lineations, segments of L-DGO tracks on which anomalies M-2 and M-4 were observed; FZ, fracture zones; ●, DSDP site numbers.

orientation just before the end of the quiet period, and swept pre-existing 'Tethyan' and Mesozoic crust, and finally its own northern flank, northwards into the Java trench (aided since about 55 Myr BP by the northward spreading of the present South-east Indian Ridge).

The orthogonal relationship between the Perth magnetic lineations and the Naturaliste fracture zone suggests that the Australian continental margin from Perth to the Wallaby plateaux was an initial line of weakness along which transform faulting occurred. This geometry also suggests that the northern margin of the Naturaliste Plateau (Fig. 1) owes its shape to initial rifting and transform faulting. If the identification of the Broken Ridge sequence as Mesozoic-age anomalies is correct, then the oldest crust in the southern Wharton basin (early to middle Jurassic) should lie between latitudes 18°–23°S just east of the Ninetyeast Ridge.

Whatever its mode of origin, the existence of the Perth sequence has important ramifications regarding the breakup of eastern Gondwanaland and the evolution of the eastern Indian Ocean. The Perth lineations are consistent with the DSDP data and stratigraphical evidence from the coastal basins of Western Australia⁷ in that they disallow a gap in Gondwanaland west of Australia, at least in late Jurassic times. The seafloor of the region of the inferred gap,

that is, the present southern Wharton Basin, is not of Tethyan vintage, but has been created since the breakup of Gondwanaland. The Perth sequence by itself provides no indication as to which continental block lay adjacent to Western Australia, but it does prove that the block began to drift away to the north-west before the formation of anomaly M-11 (127 Myr BP). The trend of the Broken Ridge and quiet zone lineations indicates that the continental block

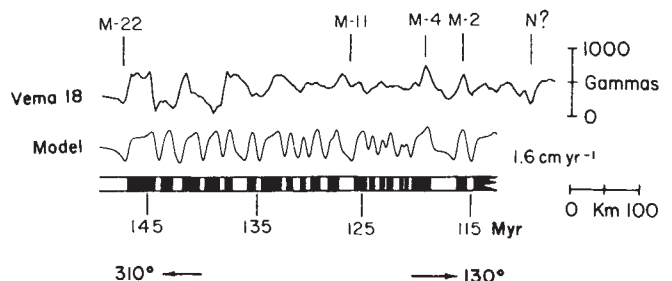


Fig. 4 Observed magnetic profile Vema 18 projected perpendicular to the strike of anomaly M-2 and plotted above the model anomaly profile and block model; the corresponding track segment is shown in Fig. 3.

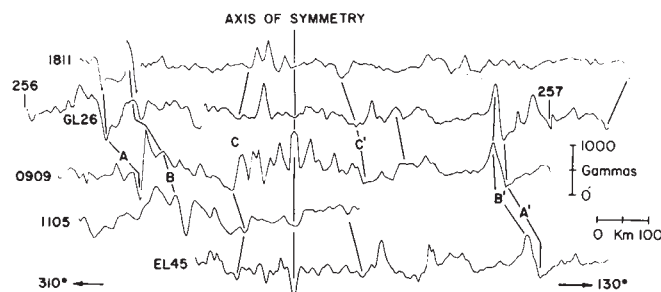


Fig. 5 Observed magnetic anomaly profiles across the proposed spreading centre in the quiet zone between the Perth and Broken Ridge sequences. The profiles are projected perpendicular to the axis of symmetry; the corresponding track segments are shown in Fig. 3. Anomalies A-A' to C-C' correlate, and may be isochronous.

in question continued to move in a north-westerly direction. The late Jurassic-late Cretaceous spreading centre postulated here indicates that the block could have reached the vicinity of longitude 90°E by the time north-south spreading began adjacent to the present Ninetyeast Ridge^{11,20}. This scenario strongly suggests that India was the continental block that abutted Western Australia.

I thank Drs Roger Larson and Dennis Hayes for discussions. This research has been sponsored by the Office of Polar Programs of the National Science Foundation.

Received February 18; revised April 29, 1974.

- ¹ DuToit, A. L., *Our Wandering Continents* (Oliver and Boyd, Edinburgh and London, 1937).
- ² Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).
- ³ Dietz, R. S., and Holden, J. C., *J. geophys. Res.*, **75**, 4939 (1970).
- ⁴ Dietz, R. S., and Holden, J. C., *Nature*, **229**, 309 (1971).
- ⁵ Smith, A. G., Briden, J. C., and Drewry, G. E., *Organisms and Continents through Time*, Spec. Paper 12 (Paleontological Association, London, 1973).
- ⁶ Sprigg, R. C., *Aust. Petrol. Explor. Ass. J.*, (1966).
- ⁷ Veevers, J. J., Jones, J. G., and Talent, J. A., *Nature*, **229**, 1 (1971).
- ⁸ Falvey, D. A., *Aust. Petrol. Explor. Ass. J.*, **12**, 86 (1972).
- ⁹ Ridd, M. F., *Nature*, **234**, 531 (1971).
- ¹⁰ Audley-Charles, M. G., Carter, D. J., and Milsom, J. S., *Nature*, **239**, 35 (1972).
- ¹¹ Slater, J. G., and Fisher, R. L., *Bull. geol. Soc. Am.*, **85**, 683 (1974).
- ¹² Larson, R. L., and Pitman, W. C., III, *Bull. geol. Soc. Am.*, **83**, 3645 (1972).
- ¹³ Larson, R. L., and Ladd, J. W., *Nature*, **246**, 209 (1973).
- ¹⁴ DSDP Scientific Staff, Leg 32, *Geotimes*, **18**, (12) 14 (1973).
- ¹⁵ Vogt, P. R., Anderson, C. N., and Bracey, D. R., *J. geophys. Res.*, **76**, 4796 (1971).
- ¹⁶ DSDP Scientific Staff, Leg 27, *Geotimes*, **18**, (4) 16 (1973).
- ¹⁷ Poehls, K. A., Luyendyk, B. P., and Heirtzler, J. R., *J. geophys. Res.*, **78**, 6985 (1973).
- ¹⁸ DSDP Scientific Staff, Leg 26, *Geotimes*, **18**, (3) 16 (1973).
- ¹⁹ DSDP Scientific Staff, Leg 22, *Geotimes*, **17**, (6) 15 (1972).
- ²⁰ McKenzie, D., and Slater, J. G., *Geophys. J. R. astr. Soc.*, **25**, 437 (1971).
- ²¹ Slater, J. G., Anderson, R. N., and Bell, M. L., *J. geophys. Res.*, **76**, 7888 (1971).
- ²² Heirtzler, J. R., *et al.*, *Science*, **180**, 952 (1973).

Gene duplication in experimental enzyme evolution

Peter W. J. Rigby*, Bruce D. Burleigh, jun.† & Brian S. Hartley‡

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

A system to study enzyme evolution experimentally has been developed. Multiple mutations are required to improve enzyme specificity and the authors propose that such mutations will be acceptable only in 'silent' gene copies—shown here to be a frequent response to selective pressure.

DIVERGENT evolution of an enzyme family from a common ancestor must involve gene duplication as the first step, followed by mutation of one copy to a 'silent gene' that produces an inactive product that cannot fold correctly¹. Natural selection will conserve the sequence of the viable copy but the progeny of the silent gene will become heterogeneous in the population by both mutation and recombination. A reversion of the original lesion or a compensating mutation may restore at any time the capacity of the protein to fold correctly and mutations accumulated in the 'silent' phase will then be expressed. Natural selection may refine the structure thereafter either back to the old specificity—whereby isoenzymes would result

—or to a new specificity, establishing an enzyme family.

Some evidence for this hypothesis can be gleaned from the tertiary structures of the serine proteinases, trypsin, chymotrypsin and elastase². The conformations of their polypeptide chains are almost superimposable and most of the internal residues are identical or chemically similar. Only one or two changes in the surface are adequate to explain their different specificities, but over 80% of the rest of the surface residues are different. Even in the highly conserved interiors there are clusters of different residues that seem to provide radically different solutions to the same space-filling problem. It is hard to see how these alternative arrangements could have evolved without going through an intermediate that could not fold correctly.

The fossil evidence that would support these evolutionary hypotheses has long since been degraded, however, and we are aware of the weakness of arguments based only on comparisons of contemporary structures—even of tertiary structures. Some discipline would be imposed on our imagination if we could observe in detail the evolution of an enzyme towards a new specificity under precisely defined selective pressure.

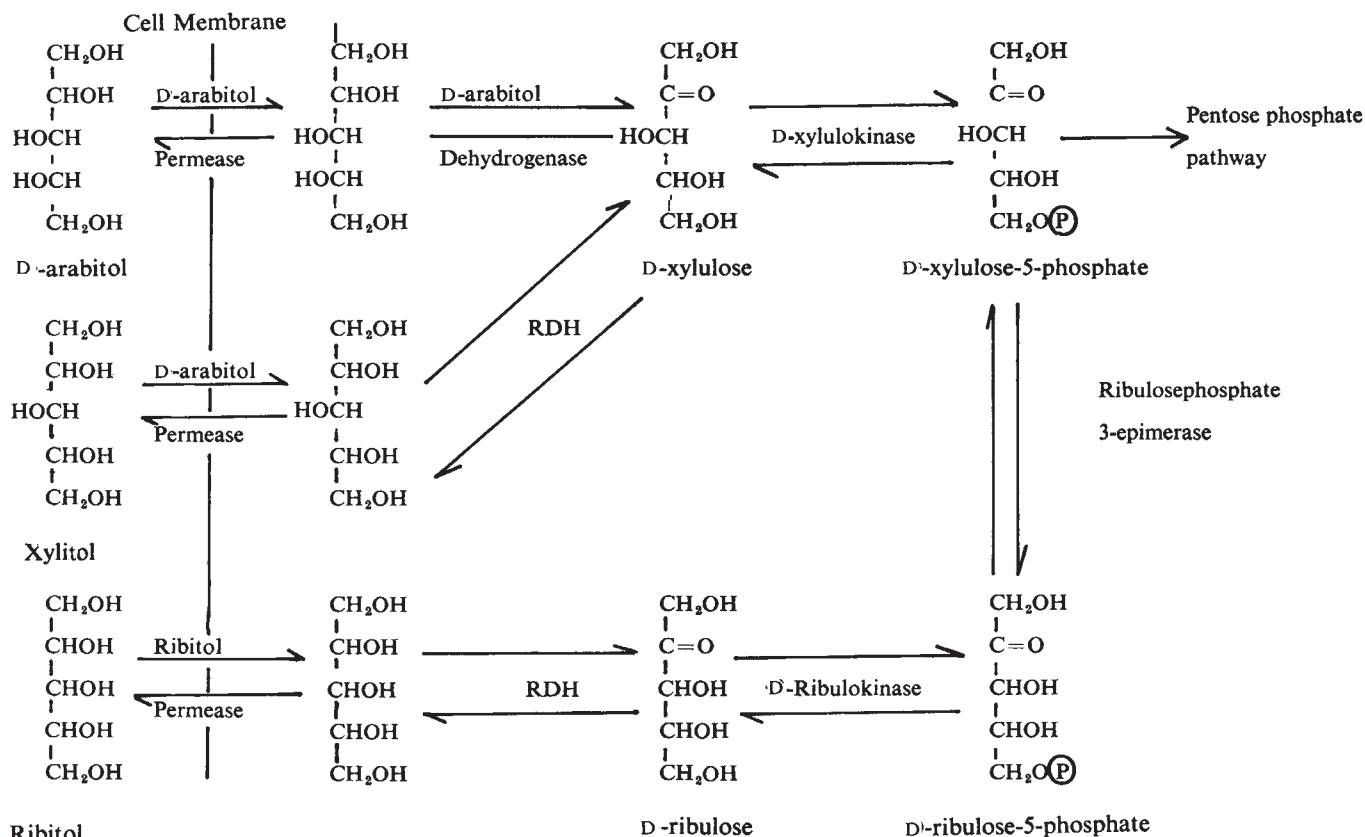
Continuous culture of microorganisms in a chemostat represents a closed ecological niche in which the biological fitness of the organism can be measured by the steady-state biomass and concentration of growth-limiting nutri-

Present addresses:

* Department of Biochemistry, Stanford University Medical Centre, Stanford, California, 94305, USA.

† Department of Biochemistry, M.D. Anderson Hospital and Tumour Institute, Texas Medical Center, Houston, Texas, 77025, USA.

‡ Department of Biochemistry, Imperial College, London SW7 2AZ.

Fig. 1 Pentitol metabolism in *Klebsiella aerogenes*.

ment in the effluent. If the growth-limiting carbon source is a poor substrate for an essential catabolic enzyme, growth may be limited by the activity of this enzyme. Mutants with increased enzyme activity will outgrow the ancestral strain and displace them from the niche³. Such an 'evolutionary step' will be signalled by a rise in steady-state biomass and a fall in effluent substrate level. Samples of the 'ancestral' and 'evolant' cultures can be stored as 'fossils' and batch cultures of these can be grown separately to examine the properties of the enzyme in each strain.

Evolution of ribitol dehydrogenase

The pioneering studies of Mortlock and Wood⁴ and of Lin⁵ and their colleagues on the growth of *Klebsiella aerogenes* on pentitols, established the metabolic pathways shown in Fig. 1. The wild type organism will grow on ribitol or D-arabitol as sole carbon source, having an inducible pentitol permease, ribitol dehydrogenase (RDH) (ribitol: NAD oxidoreductase E.C.1.1.1.1.c) and D-arabitol dehydrogenase. Xylitol and L-arabitol, which are not normally found in nature, will not support growth of the wild type organism, but mutants that are constitutive for ribitol dehydrogenase will grow slowly on these 'unnatural' pentitols. Their metabolism has been conclusively demonstrated⁶ to be by a side specificity of ribitol dehydrogenase, but whereas the apparent K_m for ribitol was about 1 mM, that for xylitol and L-arabitol was around 1 M. It was likely, therefore, that the activity of this enzyme would limit the growth of *K. aerogenes* on xylitol in continuous culture, since the pentitol permease seems to transport xylitol efficiently.

We therefore began an extensive study of the activity of ribitol dehydrogenase in continuous cultures of a constitutive strain, A, of *K. aerogenes* (strain X1 (ref. 5)). This was grown in a minimal salts medium with xylitol (0.2% w/v) as sole carbon source in a continuous culture apparatus of our own design and manufacture based on the Porton type fermenter⁷. The appearance of better adapted

strains was followed by monitoring the bacterial dry weight and residual substrate level in the chemostat.

To demonstrate that our system would select putative evolants we grew an arginine-requiring derivative of strain A on medium supplemented with arginine and guanine; at steady state the chemostat contained approximately 10^{12} cells. We then inoculated with 20 cells of a guanine-requiring derivative of strain B (strain X2 (ref. 5)) which has a mutant ribitol dehydrogenase with an apparent K_m for xylitol of 0.5 M. Twenty cells were used to avoid the statistical chance that the fitter strain would be eliminated³. Takeover was monitored by measuring normal chemostat parameters (Fig. 2a) and by plating on appropriate media (Fig. 2b); these data show that takeover was complete within 7 d.

Spontaneous evolution

Our initial experiments (summarised in Fig. 3 and Table 1) were performed without mutagenesis to mimic natural evolution. Strains A1 and A11 were investigated in detail (Table 1). The apparent maximum specific growth rates on 0.2% xylitol, measured by the washout technique of Tempest⁸, confirmed that both strains had increased fitness for their environment. The ribitol dehydrogenase activity of strains A, A1 and A11 was measured in cell free extracts obtained by ultrasonication of saturated batch cultures grown on xylitol. Neither the apparent K_m for xylitol nor the ratio of xylitol activity to ribitol activity was altered, but a significant increase in total enzyme activity occurred at each step.

Is this due to superproduction of wild type enzyme or to evolution of enzymes with increased catalytic efficiency? We purified each of these enzymes to homogeneity by the methods of Taylor, Rigby and Hartley⁹, and examined their kinetic parameters. There was no detectable change in enzyme activity (Table 1), but it is clear from the purification schedules (Table 2) that strain A1 makes five times

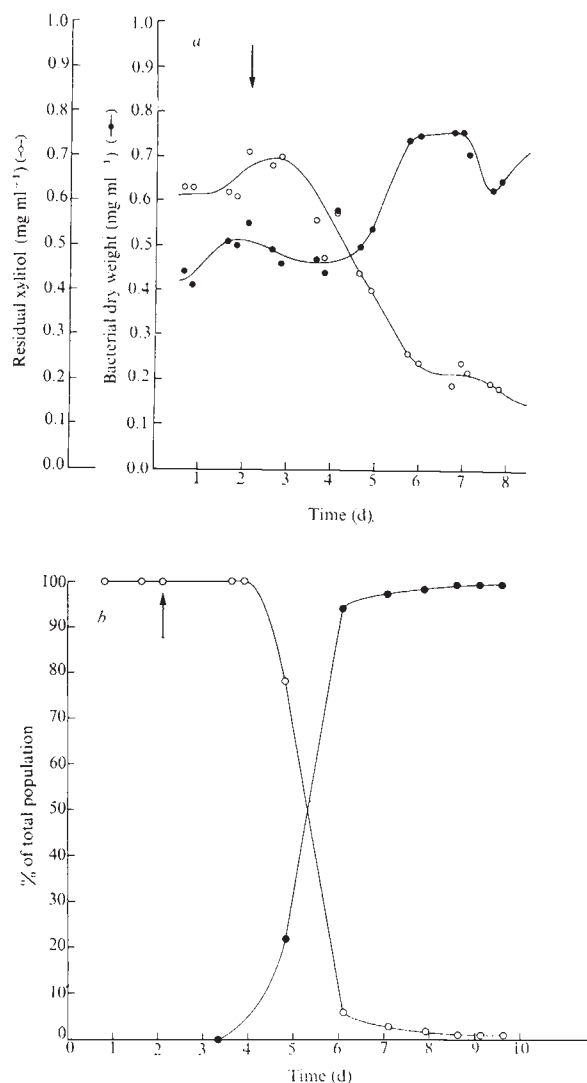


Fig. 2 *a* Chemostat parameters from control takeover experiment. ○, Residual xylitol; ●, bacterial dry weight. *b*, Differential counting of wild type and K_m mutant strains during control takeover experiment. The total population of the chemostat was approximately 10^{12} organisms. ○, Strain A; ●, strain B. At the point indicated by the arrow, about twenty cells of strain B were added to a chemostat containing a steady-state culture of strain A.

more enzyme than strain A and strain A11 makes fifteen times more. In the latter case the enzyme represents 17% of the total protein in the extract.

Spontaneous evolution gives only increased enzyme production. Will specific mutagenesis change this pattern? We built a small ultraviolet lamp into our chemostat and irradiated cultures at a low level such that about 10% of such treatments failed to initiate takeover events. The genealogy of fitter strains selected in this way is shown in Fig. 3, and Table 1 lists the properties of a typical pair. Strain A2 appeared by all criteria to be wild type, but strains A21 and A211 contained elevated levels of enzyme. The enzyme was purified to homogeneity from these strains and was kinetically indistinguishable from wild type (Table 1). Similar results were found for all the ultraviolet-induced evolvants shown in Fig. 3; 20% of the total protein of strain A6111 was ribitol dehydrogenase. Superproduction of wild-type enzyme seems to be the invariable response of the organism to this selective pressure, whether mutations arise spontaneously or are induced by low levels of mutagenesis.

Enzyme superproduction

Our original hypothesis about evolution of new enzymes demands gene-doubling as the first step. Is gene-doubling the explanation for enzyme superproduction, or are other phenomena responsible, such as promoter mutations which lead to high transcription rates for the gene? To distinguish gene-doubled strains we measured the frequency at which mutants lacking ribitol dehydrogenase occurred in strains A, A1 and A11. If the probability of a bacterium carrying a single RDH gene mutating to be RDH^- is P , then that for a bacterium carrying two copies is P^2 , for one carrying three copies P^3 and so on. This makes the reasonable assumption that each copy will be equally mutable at sites essential for enzyme function.

Parallel cultures of each strain were subjected to mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine followed by a penicillin selection against xylitol. Mutants unable to grow on xylitol but able to grow on D-xylose were isolated by replica plating; those which retained the ability to grow on ribitol, being revertants of the constitutive mutation, were discarded. This screening procedure produces a set of mutants which contains both RDH and permease negatives; these were distinguished by testing each xylitol-negative clone for ribitol dehydrogenase activity. The mutation frequency for the wild type is sufficient to guarantee against the isolation of dependent mutants.

Table 3 shows that strains A and A1 have approximately equal mutation frequencies whereas that for strain A11 must be less than the square of the wild type frequency. This suggests that strain A11 carries multiple copies of the RDH gene, whereas strain A1 does not.

This conclusion was confirmed by segregation analysis. Strains which carry two contiguous copies of a gene segregate clones at a high rate with only one copy¹⁰⁻¹³. We examined the frequency at which superproducing strains, which give large colonies on 0.05% xylitol plates, produced small colonies after overnight growth on broth. A number of these small colonies was picked and assayed for ribitol dehydrogenase activity after growth on casamino acids and ultrasonication. Table 4 shows that about one-fifth of the small clones selected from strain A11 had wild type levels of enzyme, giving a segregation frequency of 1.4×10^{-3} , whereas strain A1 produced no segregants. Reversion of a point mutation would occur at a frequency of about 10^{-6} , so strain A11 carries multiple copies of the RDH gene.

Strain A211 appears to carry more copies of the gene than strain A11, since it segregates at a higher frequency (Table 4). Some of the A211 segregants have enzyme levels like strain A11, while others are clearly wild type in behaviour (another class A11/A in Table 4, could not be

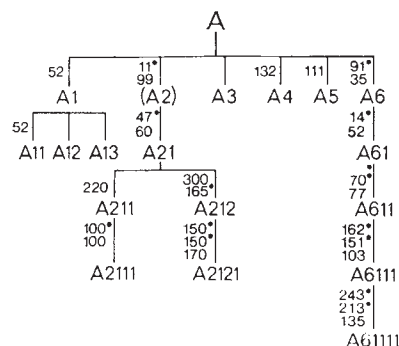


Fig. 3 Genealogy of spontaneous and ultraviolet-induced evolvants. The strain numbers indicate the evolutionary history of the strain. Thus A1 and A2 and so on are derived directly from A; A11 and A12, and so on from A1. The numbers between strains are the generations which elapsed before isolation of the new strain. *An ultraviolet mutagenesis.

Table 1 Initial experiments to mimic natural evolution performed without mutagenesis

Strain	Apparent μ_m (h ⁻¹)	Apparent K_m for xylitol in crude extract	Specific activity in crude extract (RDH units mg ⁻¹ soluble protein)	Apparent K_m for xylitol of pure enzyme	Xylitol:ribitol activity ratio for pure enzyme
A	0.53	1.2 M	2.0	1.2 M	0.24
A1	0.64	1.4 M	9.0	1.4 M	0.21
A11	0.82	1.3 M	32.0	1.3 M	0.17
A21	0.64	1.6 M	11.0	—	—
A211	0.84	1.2 M	27.0	1.3 M	0.18

One unit of RDH activity is defined as that amount which reduces 1 μ mol NAD min⁻¹ in the conditions of the standard assay system; 0.83 mM NAD, 100 mM phosphate buffer, pH 7.0, 28°C. Apparent K_m values were determined in this system. The xylitol:ribitol activity ratio is that of the apparent V_{max} on xylitol to the specific activity determined on saturating (50 mM) ribitol.

unambiguously assigned as A or A11). These results are those expected if A11 carries two copies of the RDH gene while A211 carries three copies. If A211 carried four copies, segregants with an enzyme level between A11 and A211 should have been detected.

Comparison of these segregation frequencies with results for duplications of structural genes in *Escherichia coli* suggests that strain A11 may have a duplication covering several genes. Segregation frequencies have been measured for *E. coli* strains with gene duplications of glycyl-tRNA synthetase¹⁰, glycyl-tRNA¹¹, tryosyl-tRNA^{12,14}, and the *lac* operon¹⁵ and the *rII* region of bacteriophage T4D¹³. The

The rate of spontaneous mutation to RDH⁻ for this organism is about 10⁻⁶. There are 249 residues per chain, thus 250 triplets in the gene and each triplet can yield on average six amino acid replacements by a single base change. Therefore the frequency of any particular single-step replacement will be of the order of 10⁻⁹, assuming equal mutability across the gene. Values of this order have been observed for *E. coli* genes, ranging from 2 × 10⁻¹⁰ for streptomycin resistance¹⁶ to 10⁻⁸ for bacteriophage T1 resistance¹⁷.

We have screened some 10¹⁴ wild type organisms without detecting mutational alteration of the enzyme's specificity

Table 2 Comparison of RDH purification for strains A, A1 and A11

Purification step	Total units (× 10 ⁻⁵)			Specific activity (RDH units mg ⁻¹ protein)			Purification			Yield		
	A	A1	A11	A	A1	A11	A	A1	A11	A	A1	A11
Crude extract	1.8	8.8	27.5	1.0	5.1	18.7	1	1	1	100	100	100
Streptomycin supernatant	1.6	6.0	17							92	69	62
35→55% (NH ₄) ₂ SO ₄ cut	1.0	4.5	11	2.3	18.8	36.5	2.2	3.6	1.9	59	52	39
60 mM DEAE batch eluate	0.75	4.0	8	10.3	74	119	10	14.4	6.4	44	46	29
DEAE gradient eluate	0.7	2.4	4.5	55	87	107	52	16.9	5.8	38	27	16
G-200 Sephadex eluate	0.45			83			80			26		

The figures for total enzyme units are normalised to a preparation from 1 kg of cells

segregation frequencies we observe are higher than for strains with a tandem gene duplication^{11,14,15}, but lower than for strains in which 5% of the genome is duplicated¹⁰. This supposition is supported by the observation that during enzyme purification from strain A11, a second protein was observed to be increased considerably in amount relative to strain A, and acrylamide gels of crude extracts of A11 showed that this protein and ribitol dehydrogenase were present in roughly equal amounts.

Why make more enzyme?

We have shown that enzyme superproduction is an invariable response to the selective pressure used under conditions of mild mutagenesis, whether spontaneous or induced by ultraviolet light or nitrosoguanidine (J. M. Douthie and B. S. H., unpublished). Figure 2 clearly demonstrates that a mutant with increased specificity for xylitol such as strain B (obtained after powerful nitrosoguanidine mutagenesis⁹) would have taken over from strain A in our chemostats. Moreover, the growth rate of strain B on xylitol is the same as that of strains A1 and A21, so it would compete effectively with a first-stage superproduction mutant. This phenomenon is not an artefact of chemostat selection since we have also found nothing but superproducers by plate selection on low concentrations of xylitol after mild mutagenesis. One must conclude that the frequency of mutations leading to increased enzyme production is much greater than that leading to improved enzyme specificity.

for xylitol. If such mutational improvement could result from a single base change it would occur at a frequency greater than 10⁻¹⁰. Statistical considerations allow that some mutations will not be detected in the chemostat³, but we calculate that we should detect around 10% and certainly more than 1% of all events. We conclude that no point mutation can lead to a viable enzyme with improved specificity for xylitol.

We are forced to conclude that this ribitol dehydrogenase cannot evolve towards a new specificity for xylitol by single step mutations whilst under continuous selective pressure. Multiple mutations may, and do (J. M. Douthie and B. S. H.,

Table 3 Determination of mutation frequencies

Strain	No. of xylitol negative clones	No. of RDH negative clones	Frequency of RDH negative clones
A	71	43	2.6 × 10 ⁻⁴
A1	30	18	6.0 × 10 ⁻⁴
A11	0	0	< 3.0 × 10 ⁻⁸

Each strain was grown on B broth to a density of 2 × 10⁸ cells ml⁻¹ then washed with and resuspended in Tris-maleate, pH 6.0; nitrosoguanidine was added to a final concentration of 0.2 mg ml⁻¹ and the culture incubated at 37°C for 30 min. The cells were then washed with B broth, diluted 1:20 into the same medium and grown out overnight. After being washed with M9 and then starved for 20 min the culture was diluted with M9 to give a density of 10⁷ cells ml⁻¹. Xylitol was added to 0.2% and benzylpenicillin to 0.6 mg ml⁻¹ and the culture was incubated for 4 h. Appropriate dilutions were spread on to M9/xylitol plates which after incubation were replicated on to M9/xylitol plates.

Table 4 Results of segregation experiments

Strain	Small clones (%)	%	Segregants No.	Class
A1	0.03	0	0	—
A11	0.76	0.14	28	{ A11 (23) A (5)
A211	0.75	0.68	32	{ A211 (3) A11 (13) A11/A (9) A (7)

After being cloned several times on xylitol, strains were grown on B broth until the optical density at 650 nm was approximately 1.0. Aliquots of a suitable dilution were spread on to M9/0.05% xylitol plates which after incubation were examined and the number of small colonies determined. A sample of these small colonies was picked and assayed for RDH level. Cultures were grown on 3% casamino acids and the cells were ruptured by treatment with lysozyme/EDTA and Triton X-100. This procedure is as effective at releasing RDH as ultrasonication. Extracts were then assayed for RDH activity and total protein and from the specific activities thus determined each clone could be classified.

unpublished), produce enzymes with improved specificity, but these would occur spontaneously at a very low frequency—around 10^{-18} . Spontaneous steps which increase enzyme level, however, are more frequent—around 10^{-13} —and most of these steps are due to gene multiplication. Thus far, therefore, these experimental results are in accord with the evolutionary hypothesis propounded above. Further experiments are needed to test the evolutionary pathway of the gene-doubled strains under conditions of fluctuating selective pressure.

If it is generally true that multiple mutations are required for evolutionary improvement, then it is likely that such mutations will often be incompatible with the tertiary structure of the protein. Evolutionary progress and viability can be reconciled by gene duplication, which allows the second gene copy to accumulate mutations while not immediately constrained by natural selection. We have demonstrated that multiple mutations are required for, and gene duplications are a frequent part of, one evolutionary pathway.

Opposite conclusions can be drawn, however, from the studies¹⁸ of Clarke and her collaborators on the experimental evolution of an amidase in *Pseudomonas aeruginosa*. Whereas the wild type enzyme is specific for acetamide

and propionamide, new specificities for butyramide, valeramide, phenylacetamide and acetanilide evolve readily by single mutations. We suspect that this apparent contradiction is explained by the fact that we are trying to set a more difficult evolutionary problem for a more complex enzyme: a change in stereospecificity about the carbon atom adjacent to the site which donates hydride ion to NAD. But the contrast between the conclusions which we draw strongly indicates that the evolutionary potential of an enzyme may itself be a function of its tertiary structure.

We thank Sydney Brenner for drawing this system to our attention and for valuable discussion, Chris Bruton for much helpful advice and Denis Herbert for the hospitality of his laboratory and an introduction to chemostat technology. B. D. B. is grateful for the award of a Post-doctoral Fellowship by the American Cancer Society, while during part of this work P. W. J. R. held a Scholarship from the Medical Research Council.

Received July 27, 1973; revised June 18, 1974.

- Hartley, B. S., *et al.*, *FEBS Symp.*, **29**, 151–176 (North Holland, London, 1972).
- Hartley, B. S., *Phil. Trans. R. Soc. Lond.*, **B257**, 77–87 (1970).
- Powell, E. O., *J. gen. Microbiol.*, **15**, 492–511 (1956); **18**, 259–268 (1958).
- Mortlock, R. P., Fossitt, D. D., and Wood, W. A., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 572–579 (1965).
- Lerner, S. A., Wu, T. T., and Lin, E. C. C., *Science*, **146**, 1313–1315 (1964).
- Wu, T. T., Lin, E. C. C., and Tanaka, S., *J. Bact.*, **96**, 447–456 (1968).
- Herbert, D., Phipps, P. J., and Tempest, D. W., *Lab. Practice*, **14**, 1150–1161 (1965).
- Tempest, D. W., in *Methods in Microbiology* (edit. by Norris, J. R., and Ribbons, D. W.), **2**, 259–276 (Academic Press, London, 1970).
- Taylor, S. S., Rigby, P. W. J., and Hartley, B. S., *Biochem J.* (in the press).
- Folk, W. R., and Berg, P., *J. molec. Biol.*, **58**, 595–610 (1971).
- Hill, C. W., Foulds, J., Soll, L., and Berg, P., *J. molec. Biol.*, **39**, 563–581 (1969).
- Russell, R. L., Abelson, J. N., Landy, A., Geftter, M. L., Brenner, S., and Smith, J. D., *J. molec. Biol.*, **47**, 1–13 (1970).
- Parma, D. H., and Snyder, M., *Genetics*, **73**, 161–183 (1973).
- Miller, R. C., Besmer, P., Khorana, H. G., Fiant, M., and Szybalski, W., *J. molec. Biol.*, **56**, 363–368 (1971).
- Horiuchi, T., Horiuchi, S., and Novick, A., *Genetics*, **48**, 157–169 (1963).
- Newcombe, H. B., and Hawkins, R., *J. Bact.*, **57**, 565–572 (1949).
- Luria, S. E., and Delbruck, M., *Genetics*, **28**, 491–511 (1943).
- Betz, J. L., Brown, P. R., Smyth, M. J., and Clarke, P. H., *Nature*, **247**, 261–264 (1974).

letters to nature

Black holes of small mass

ANY star which has exhausted all of its nuclear fuel and which cannot eject sufficient matter so that its mass becomes less than roughly two solar masses, must undergo gravitational collapse to become a black hole. Thus one usually associates black holes with masses of stellar proportions. But it is possible that during the initial chaotic conditions of the Universe, black holes of much smaller mass^{1,2}, down to 10^{-5} g, may have been formed. Here we suggest an experiment by which one may detect or set meaningful upper limits to the number density of black holes of small mass (hereinafter referred to as BHSM).

First we estimate the BHSM–Earth collision time, τ , under reasonable assumptions, for various values of the mass m of

the BHSM. Assume the space density of BHSM is ρ g cm⁻³. Because the mass distribution of the BHSM is unknown, we may assume that they all have the same mass m , and investigate the consequences of varying the mass. Then³,

$$\tau^{-1} = \pi R_E^2 \rho V [1 + (V_e/V)^2]/m \quad (1)$$

where R_E is the radius of the Earth, V_e ($\approx 10^6$ cm s⁻¹) is the escape velocity from the Earth, and V the velocity of the BHSM at infinity. Because of the solar motion⁴ and the Earth's rotation, $V \gtrsim 10^6$ cm s⁻¹. So $(V_e/V)^2 \lesssim 1$ and

$$\tau \approx m/\pi R_E^2 \rho V \approx 10^{-10} m (10^7/V) (10^{-23}/\rho) \text{ yr} \quad (2)$$

The parameter m may be varied in order to estimate the

maximum possible flux of BHSM and to ascertain whether such a flux is detectable.

Highly sensitive superconducting accelerometers^{5,6} are now being developed for use in low temperature gravitational wave detectors. One of us (D.G.B.) is constructing an accelerometer which is also particularly applicable to the detection of BHSM. This accelerometer measures the deflection of a magnetically levitated test object of mass M against a constraining magnetic field. Deflections Δx as small as 10^{-17} cm may be detected. To detect a BHSM we must observe a differential acceleration of the test mass relative to its support. Suppose the accelerometer is supported by a rigid body so that the test mass is at a distance l from the centre of mass C . Consider the passage of a BHSM of mass m with speed V_0 at a distance s from the test mass and $(s+l)$ from C . Then, neglecting geometrical factors, the test mass gains an impulsive velocity⁷ $2Gml/V_0 s(l+s)$ relative to C . Because the test mass is attached to the accelerometer by a magnetic 'spring' of 'spring constant' $M\omega^2$

$$\frac{1}{2} M \omega^2 (\Delta x)^2 = \frac{1}{2} M [2Gml/s(l+s)V_0]^2$$

so that

$$m = V_0 \omega (\Delta x) s(l+s)/2Gl \quad (3)$$

We note that the mass on which the accelerometer is mounted need not be a gravity wave antenna, and that ω is not related to the frequency of the antenna. The strength of the magnetic field may be varied to vary the spring constant $M\omega^2$. The value of ω can vary from 10^{-2} rad s⁻¹ to 10^4 rad s⁻¹. For greatest possible sensitivity, we take $\omega = 10^{-2}$ rad s⁻¹ and $\Delta x = 10^{-17}$ m.

For BHSM to be observable, we require a flux sufficient to produce one event per year. Setting $\tau R_E^2/ns^2 = 1$ yr in equation (2) we require that

$$m \leq 2.4 \times 10^{-8} n s^2 (\rho/10^{-23}) (V/10^7) \quad (4)$$

where n is the number of detectors.

For simplicity we separate two cases, $s \leq l$ and $s > l$. If $s \leq l$, equation (3) reduces to $m = \omega(\Delta x)sV_0/2G$ to within a factor of two. Now if $V > V_c$ it follows that $V_0 \sim V$. So to detect a BHSM travelling within a distance s it is necessary that

$$m \gtrsim 7.5 \times 10^{-6} s (V/10^7) \text{ g} \quad (5)$$

Inequalities (4) and (5) can both be satisfied only if

$$s \gtrsim (3.1 \times 10^3/n) (10^{-23}/\rho) \text{ cm}$$

If the apparatus is to be isolated, l is restricted to about 10^3 cm. Taking $s \leq 10^3$ cm and choosing $n = 100$ it follows that

$$2 \times 10^{-5} (10^{-23}/\rho) (V/10^7) \leq m \leq 2(\rho/10^{-23}) (V/10^7) \quad (6)$$

So the range of masses that can be detected depends on ρ and V . We note also that equation (6) can be satisfied only if $\rho \gtrsim 10^{-25}$ g cm⁻³. It is likely that during the early history of the Universe BHSM became concentrated into galaxies with a space density ρ_G . An upper limit⁸ for ρ_G in the solar neighbourhood is about 10^{-23} g cm⁻³. If ρ_G equals this upper limit and $V \sim 10^7$ cm s⁻¹, masses in the range 10^{-5} to 1 g may be detected.

If the Earth itself is the supporting mass $l = R_E$. Taking an upper limit for s of 10^8 cm one requires that $\rho \gtrsim 10^{-30}$ g cm⁻³. If ρ_c be the cosmological space density of BHSM then⁹, $\rho_c \lesssim 10^{-28}$ g cm⁻³. If this upper limit is attained and $V \sim 10^7$ cm s⁻¹, masses in the range 1 to 10^9 g may be detected. In this case narrow band filtering of the seismic noise at the frequency corresponding to the interaction time of the BHSM would be necessary in order to reduce the noise level. Such filtering could be difficult.

For the case $s > l$, from equation (3) we have that $m \approx \omega(\Delta x)V_0 s^2/2Gl$ to within a factor of two, so that masses in the range

$$\begin{aligned} 7.5 \times 10^{-6} (V/10^7) s^2/l &\leq m \leq \\ 2.4 \times 10^{-8} ns^2 (\rho/10^{-23}) (V/10^7) &\end{aligned} \quad (7)$$

may be detected provided $\rho/10^{-23} \gtrsim 3.1 \times 10^3/nl$. For example if $n = 100$, $l = 10^3$, $V = 10^7$ cm s⁻¹ and $s > 10^3$ cm, BHSM can be detected if $\rho \gtrsim 10^{-26}$ g cm⁻³.

We now consider whether BHSM can be concentrated within the Solar System through gravitational interaction with a planet such as Jupiter. This could give rise to an even larger flux of BHSM. The collision time τ_i of a BHSM with Jupiter is obtained by multiplying equation (2) by $(R_E/R_J)^2$ where R_J (≈ 0.3 AU) is the radius of the sphere of influence⁹ of Jupiter. So

$$\tau_i = 2 \times 10^{-18} m (10^7/V) (10^{-23}/\rho_G) \text{ yr} \quad (8)$$

Thus frequent collisions are possible for reasonable parameters in equation (9). Suppose that a (net) fraction α (V) of BHSM coming within the sphere of influence of Jupiter are captured by the Solar System. The contribution of these BHSM to the solar system density is

$$\begin{aligned} \rho_s &= \alpha (5 \times 10^9/\tau_i) [3 m/4\pi (7.5 \times 10^{13})^3] \\ &\approx \alpha (V/10^7) (\rho_G/10^{-23}) \times 10^{-15} \text{ g cm}^{-3} \end{aligned}$$

The value of $\alpha(V)$ is difficult to estimate, but, if $\alpha \gtrsim 10^{-8}$ some increase in ρ_s over ρ_G can be obtained. But $\rho_s \lesssim 10^{-18}$ g cm⁻³, since otherwise perturbations of spacecraft would have been discovered. It has been suggested¹⁰⁻¹² that the Tunguska meteoric event was caused by a black hole of mass $\sim 10^{21}$ g. We note that even if $\rho_s = 10^{-18}$ g cm⁻³, such an event was an extremely rare occurrence (equation (2)).

The trajectory of a BHSM can be roughly estimated by utilising the uniaxial directional properties of the accelerometer^{5,6}. Two detectors properly oriented could distinguish between particles moving up or down. Since BHSM can travel through the Earth whereas meteors cannot, those particles travelling upwards must be black holes.

We thank Drs T. P. Bernat, W. O. Hamilton and S. D. Verma for discussions.

D. G. BLAIR
G. CHANMUGAM

Department of Physics and Astronomy,

J. S. DRILLING
T. D. FAÏ, JUN.
JAMES G. PETERS

Louisiana State University Observatory,
Louisiana State University,
Baton Rouge, Louisiana 70803

Received February 1; revised May 20, 1974.

- ¹ Misner, C. W., in *Batelle Rencontres in Mathematics and Physics* (edit by De Witt and Wheeler), (Benjamin, New York, 1968).
- ² Hawking, S., *Mon. Not. R. astr. Soc.*, **152**, 75-78 (1971).
- ³ Eddington, A. S., *The Internal Constitution of the Stars*, 391 (Dover New York, 1959).
- ⁴ Delhaye, J., in *Galactic Structure* (edit by Blaauw, A. and Schmidt, M.), (Univ. Chicago Press, Chicago, 1965).
- ⁵ Logan, J. L., *Physics Today*, **26**, 44-52 (1973).
- ⁶ Boughn, S. P., Fairbank, W. M., McAshan, M. S., Paik, H. J., Taber, R. C., Bernat, T. P., Blair, D. G., and Hamilton, W. O., *Proc. IAU Symp. No. 53*, (in the press).
- ⁷ Ruderman, M. A., and Spiegel, E. A., *Astrophys. J.*, **165**, 1-15 (1971).
- ⁸ Oort, J. H., in *Galactic Structure*, (edit by Blaauw, A. and Schmidt, M.), (Univ. Chicago Press, Chicago, 1965).
- ⁹ Blanco, V. M., and McCuskey, S. W., *Basic Physics of the Solar System*, 261 (Addison Wesley, 1961).
- ¹⁰ Jackson, A. A., and Ryan, M. P., *Nature*, **245**, 88-89 (1973).
- ¹¹ Jackson, A. A., *Nature*, **245**, 397 (1973).
- ¹² Eardley, D. M., *Nature*, **245**, 397 (1973).

Particle acceleration in planetary magnetospheres

MEASUREMENTS of energetic particle fluxes in space^{1,2} have stimulated interest in the problem of particle acceleration inside planetary magnetospheres. Suggestions that terrestrial auroral (keV) electron precipitation is associated with transient space

charge effects³, have been discarded, largely because the parallel (plasma) electrical conductivity ($\sigma_{\parallel}$) is so large that magnetospheric field lines are constrained to remain equipotentials⁴.

Here I point out that planetary magnetospheric field lines are not constrained to remain equipotentials when the plasma conductivity ($\sigma_{\parallel}$) is dominated by current driven micro-instabilities as opposed to electron-ion collisions. For example in the 'collision-free' regime defined by $c_s \leq u_{\parallel} \leq v_{Te}$ and $T_e \gg T_i$, the plasma conductivity $\sigma_{\parallel}$ is determined by the spectrum of unstable ion-sound waves. The measured value is⁵

$$\sigma_{\parallel} \approx \omega_{pe} (4\pi \times 10^8) \text{ mho m}^{-1} \quad (1)$$

$c_s \equiv \sqrt{(kT_e/M_i)}$ = ion-sound speed, $v_{Te} \equiv \sqrt{(2kT_e/m_e)}$ = electron thermal velocity, $u_{\parallel}$ = relative drift velocity between the electron and ion populations, $\omega_{pe} \equiv \sqrt{(n_e e^2 / \epsilon_0 m_e)}$ = electron plasma frequency.

If $n_e \sim 10^8 \text{ m}^{-3}$, $T_e \sim 10 \text{ eV}$ and $u_{\parallel} \sim v_{Te}/20$ (where $u_{\parallel}$ is induced by transient space charges) then the associated electric field $E_{\parallel}^{(0)} = en_e u_{\parallel} / \sigma_{\parallel} \sim 4 \times 10^{-3} \text{ Vm}^{-1}$. For a flux tube or magnetic field line of length $l \sim 5 \times 10^8 \text{ m}$ (such as a Jovian dipole field line at $L \approx 3$) the parallel potential $\phi_{\parallel}^{(0)}$ between the equatorial and ionospheric regions is very high, $\phi_{\parallel}^{(0)} \approx E_{\parallel}^{(0)} l \sim 10^6 \text{ V}$.

The low parallel electron mobility (equation (1)) is the result of resonances between the current carrying electrons and the unstable, current driven, ion-acoustic modes⁶; in fact an 'effective' collision frequency (Ω), due to the turbulent electrostatic ion-sound fluctuations, can be defined where⁶

$$\Omega = (2\pi e^2 / u_{\parallel} \epsilon_0 n_e m_e^2) \Sigma(\mathbf{k}/k^2) W_{\mathbf{k}} \int d^3 \mathbf{v} \mathbf{k} \cdot (\partial f_e / \partial \mathbf{v}) \delta(\omega - \mathbf{k} \cdot \mathbf{v}) \quad (2)$$

$W_{\mathbf{k}}$ = electrostatic energy per unit volume in the mode $\mathbf{k}$, $\delta(\omega - \mathbf{k} \cdot \mathbf{v})$ indicates wave-particle resonance, the rest of the notation is standard. Because ion-sound turbulence is not isotropic, however, a small fraction δf_e of the electron population is not in resonance with the unstable modes and consequently it is not included in the summation (2). The number density of this component for a 'shifted' Maxwellian

$$f_e = n_e (\beta/\pi)^{3/2} \exp - \beta [v_{\perp}^2 + (v_{\parallel} - u_{\parallel})^2]$$

(with $\beta = m_e / 2kT_e$) is

$$\begin{aligned} \delta n_e &= \int d^3 \mathbf{v} \delta f_e \\ &= n_e (\beta/\pi)^{3/2} \int_0^{2\pi} d\theta \int_{-\infty}^{+\infty} dv_{\parallel} \int_0^{v_{\parallel}} dv_{\perp} \exp - \beta [v_{\perp}^2 + (v_{\parallel} - u_{\parallel})^2] \\ &\approx (n_e/2) (c_s/u_{\parallel})^2 \end{aligned} \quad (3)$$

(with $\alpha \equiv \sin^{-1}(c_s/u_{\parallel})$).

Assuming that the turbulent electric field $E^{(1)}$ experienced by the nonresonant electron component δf_e is stochastic, their motion can be described by a Fokker-Planck equation. Further, provided that $\langle E^{(1)}(r_0(t), t) \rangle = 0$, ($r_0(t)$ = unperturbed particle trajectory and $\langle \rangle$ indicates an ensemble average) the Fokker-Planck equation can be reduced to a simple diffusion equation

$$\{ \partial / \partial t + v_{\parallel} \partial / \partial r - (e/m_e) E_{\parallel}^{(0)} \partial / \partial v_{\parallel} \} \delta f_e = \partial / \partial v_{\parallel} (D \partial / \partial v_{\parallel} (\delta f_e)) \quad (4)$$

(D = velocity space diffusion coefficient due to the turbulent fluctuations).

It is apparent from equation (4) that the nonresonant component δf_e retains its high parallel mobility and in spite of the turbulence these electrons essentially free fall in the large electric potentials $\phi_{\parallel}^{(0)}$.

The combination of high parallel electric potentials $\phi_{\parallel}^{(0)}$ (due to 'anomalously' low conductivity effects) and a tenuous but highly mobile electron component δn_e (due to the nonisotropic nature of ion-sound turbulence) provides an important mechanism for generating sporadic and stormy outbursts of energetic electrons inside planetary magnetospheres.

M. J. HOUGHTON

Department of Geophysics & Planetary Physics,
School of Physics, University of Newcastle-upon-Tyne,
Newcastle-upon-Tyne NE1 7RU, UK

Received March 1; revised April 16, 1974.

¹ Simpson, J. A., *et al.*, *Science*, **183**, 306 (1974).

² Simpson, J. A., Eraker, J. H., Lamport, J. E., and Walpole, P. H., *Science*, **185**, 160 (1974).

³ Chamberlain, J. W., *Astrophys. J.*, **134**, 401 (1961).

⁴ Parker, E. N., *Physics of Geomagnetic Phenomena*, II, Chapter VI (Academic Press, New York, 1967).

⁵ Hamberger, S. M., and Jancarik, J., *Phys. Rev. Lett.*, **25**, 999 (1970).

⁶ Wesson, J., and Sykes, A., *Phys. Rev. Lett.*, **31**, 449 (1973).

High power acoustic radar

The range and sensitivity of turbulence detection by acoustic probing of the lower atmosphere initiated by McAllister *et al.*¹ has in part been restricted by the use of low power acoustic pulses (generally $\sim 100 \text{ W}$). We shall briefly describe a pulsed high power (8.1 kW input) sound

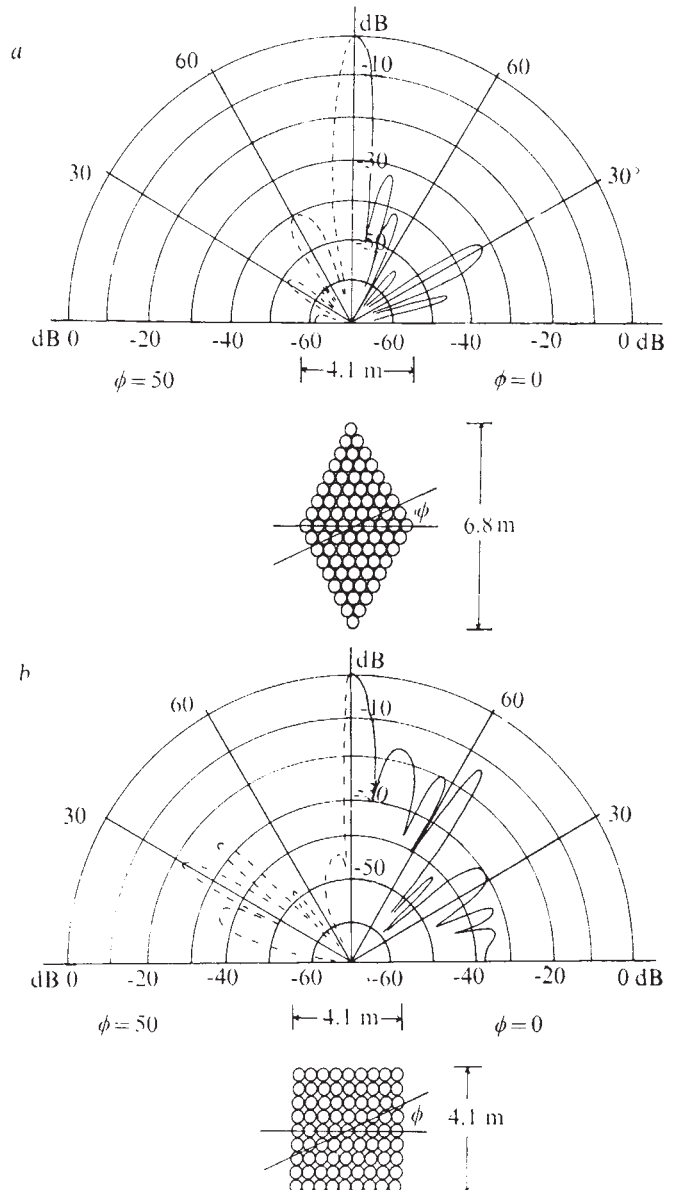


Fig. 1 Radiation patterns for, a, diamond and, b, rectangular arrays at 1,305 Hz. The solid curve represents half the cross section at $\phi = 0^\circ$ and the dotted curve is half the cross section at $\phi = 50^\circ$.

radar system and present representative data obtained with a 2.7 kW system.

An all-weather capability coupled with a high degree of system reliability and flexibility was essential. The limitations² to an acoustic radar and cost estimates led to a suitable operation frequency of approximately 1 kHz.

A high gain array was selected as the transmitter antenna. An individual transmitter array element consisted of a bin, resonant at a suitable frequency fed by an inverted 100 W electrodynamic transducer exponential horn combination (approximately 50% efficient). These transducers had proven reliabilities and the unit inversion provided the necessary transducer weather protection.

Various array configurations were numerically simulated to determine an optimum narrow beamwidth or gain and side-lobe level appropriate for an acceptable amount of noise pollution. An 81 element diamond-shaped array was finally selected and its beam pattern, shown in Fig. 1, indicates that this design has better side-lobe characteristics than a conventional rectangular array. The fact that the beam pattern is not symmetrical is not of any serious consequence. Note that only two cross sections are shown and that the patterns presented consist of the calculated space factor (array beam pattern) multiplied by the measured radiation pattern of an individual array element. The actual radiation pattern of the array has not yet been measured and effects such as atmospheric turbulence upon the beamshape are unknown. To further improve side-lobe levels, the entire transmitting array was placed in a hole 8 feet deep. Each array element will finally be driven by a separate 100-W audio amplifier, giving a total power input of 8.1 kW. The results presented here were obtained with a power input of 2.7 kW.

The receiving system uses a fibreglass parabolic dish, 5 feet in diameter, incorporating a transducer-horn element as the primary feed. This provides a broad receiving beamwidth and thus lessens signal loss due to translation of the sound pulsed by the mean wind profile. The dish was placed in a separate hole 8 feet deep (located 40 feet from the centre of the transmitter array) to isolate

the receiver from wind noise and general background surface interference. A low noise preamplifier and narrow-band tuned receiving system was used to amplify the acoustic returns and after detection a signal proportional to the logarithm of the detected signal was used to intensity modulate a chart record. Provision was made to vary the sensitivity of the receiving system as a function of time after the transmitted pulse, thus reducing the range of intensities that need be recorded.

The system was located in flat open country at Laverton, Victoria, which is traversed by the easterly flow of high pressure and cold front systems which pass across southern Australia. The results presented provide a typical cross section of records obtained during the period from mid December 1973 to mid February 1974, when the frequency of operation was 1,350 Hz, the bandwidth 5 Hz, and the pulse length 0.4 s.

The record shown in Fig. 2a was obtained after a cold front had passed the site. The development of rising echo structures is a characteristic typically associated with a cold front; in this example the highest echo is observed at a range of 9,500 feet at 0000 EST. From 0330 EST, at 7,000 feet an echo associated with subsidence inversion conditions is apparent. The arrowed bands represent periods during which the noise created by rainfall saturated the receiving system. Conditions during the approach of the centre of the following high pressure system are presented in Fig. 2b. The existence of a high degree of turbulence between the ground and the subsidence inversion is clearly indicated even during the night. From 0800 EST with the advent of surface heating, plume activity developed which was capped by the inversion at 4,300 feet.

A brief assessment of the relative merit of a 100-W input acoustic sounder compared with the high power sounder is shown in Fig. 2c. The system parameters lead to the high power radar having a signal-to-noise ratio (voltage) gain of approximately 10 over the low power sounder, and for the purposes of demonstration, the whole system was switched between power inputs of 100 W and 2.7 kW. Every 2 h the system operated in the low power

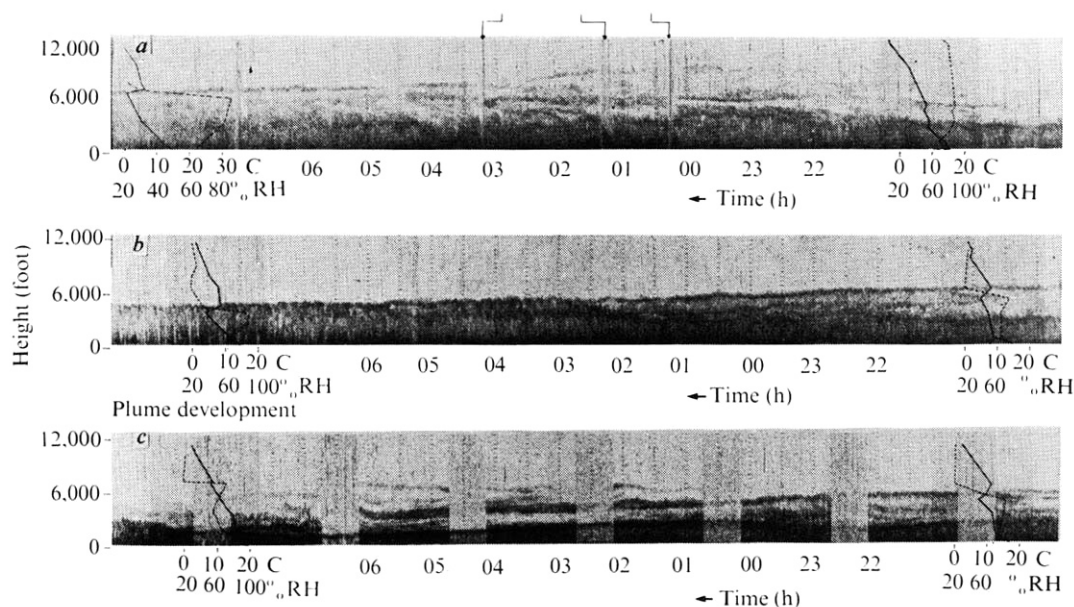


Fig. 2 High power acoustic radar record; *a*, obtained after the passage of a cold front on December 16, 1973; *b*, showing night-time turbulent structure during subsidence conditions on December 18, 1973. *c*, Record demonstrating the increased sensitivity of the 2.7-kW input acoustic radar in comparison with the 100-W acoustic radar on February 9, 1974. The abscissa represents time (increasing from right to left); the ordinate represents echo range and the intensity represents the strength of the acoustic return. Every half hour range marks at intervals of 500 feet are superimposed upon the record. Standard 0815 EST and 2015 EST Weather Bureau radiosonde temperature (solid line) and relative humidity (RH) (dotted line) profiles have also been placed on the records for comparison.

mode for a half-hour period (as from 0215 to 0245 EST). This record obtained during high pressure conditions clearly shows a substantial echo regime not indicated by the lower power unit.

These preliminary results clearly demonstrate the increased potential of high power acoustic probing. The extension to full power of 8 kW will allow the sounder to be used for high resolution acoustic sounding as well as for studying the effects of nonlinear sound propagation in the atmosphere. By measuring the change in reflection of electromagnetic energy due to the high power sound slightly altering the dielectric constant of the air, the derivation of temperature profiles is also feasible.

This work was supported in part by an ARGC grant and T.D.K. received financial assistance from a Commonwealth Postgraduate Award. We thank the Commonwealth Bureau of Meteorology for the use of their radiosonde data.

I. A. BOURNE

T. D. KEENAN

Department of Physics (RAAF Academy),
University of Melbourne,
Parkville 3052,
Melbourne, Australia

Received May 13; revised July 1, 1974.

¹ McAllister, L. G., Pollard, J. R., Mahoney, A. R., and Shaw, P. J. R., *Proc. Instn elect. electron. Engrs*, **57**, no. 4, (April 1969).

² Little, C. G., *Proc. Instn elect. electron. Engrs*, **57**, 571-578 (1969).

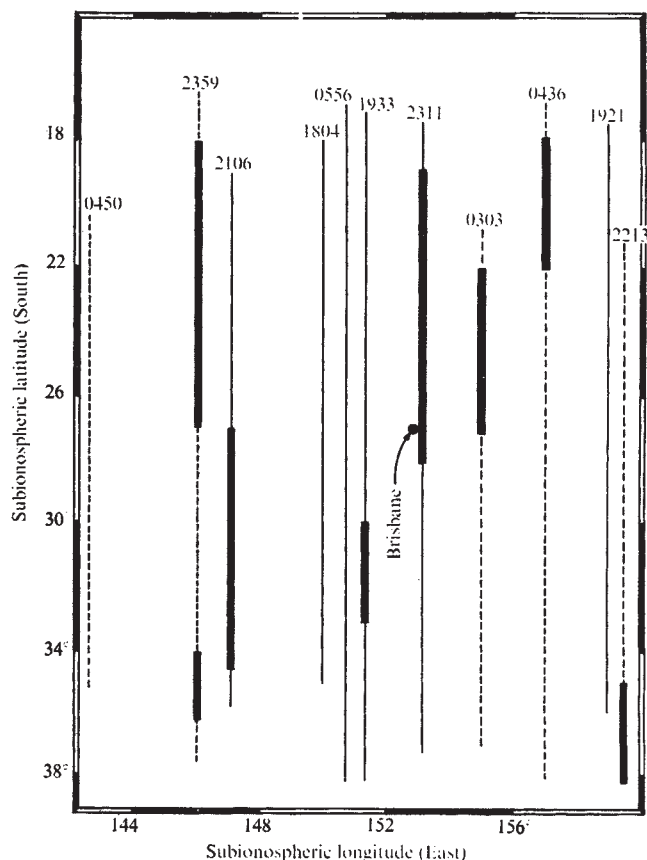


Fig. 1 Subionospheric orbits of NNSS satellites. Solid line, Night of December 7-8, 1973; broken lines, night of January 8-9, 1974; thick solid lines provide an indication of the horizontal extent of the scintillations. The time of the nearest satellite approach (150° EST) is indicated for each pass (LT).

Diurnal change in the location of scintillations in the F-region of the ionosphere

FIELD-aligned irregularities in the F-region of the ionosphere cause amplitude and phase scintillations of satellite radio transmission¹⁻⁵. It seems that scintillations increase in magnitude and number towards the northern and southern auroral zones. The latitudinal variation is, however, masked by the enhancement of scintillations whenever the angle between the ray path and the magnetic field—the aspect angle—is small. Thus, at recording stations from subauroral^{4,6} to low² latitudes, strong scintillations are observed towards the equator.

So far, studies of the amplitude and phase of the scintillations have used VHF beacon transmissions from one or two satellites, and, consequently, consecutive radio scans of the local ionosphere have been relatively infrequent. It has therefore been difficult to trace changes in the location of regions of scintillation, at short time intervals. Here, I discuss the advantages of using a multisatellite radio transmission system for detecting changes in the location of scintillations at night.

The study was based on recordings of the amplitude of radio signals from five United States Navy Navigational Satellites (NNSS) transmitting at a frequency of 150 MHz. The satellites move along polar orbits and provide frequent radio scans of the local ionosphere. The recording station was situated at Brisbane (27°S, 152.9°E; and 35.8°S geom. lat.). The receiving antenna had a broad, isotropic beam-width pattern which made it possible to record satellite passes at high zenith angles of up to 90°. As amplitude scintillations at Brisbane are mainly a night time phenomenon^{1,2} the recording period was largely limited to a time interval from 1800 to 0500 LT. It was possible to record, on average, seven NNSS passes during this time interval. Between November 22, 1973 and January 16, 1974, 347 satellite passes were recorded.

Consecutive radiosatellite scans of the local ionosphere at a height of 400 km were obtained on two nights: December 7-8, 1973 and January 8-9, 1974 (Fig. 1). It can be seen that the passes recorded at 1804 and 1921 LT on the first night did not reveal any scintillation activity. A pass at 1933 LT indicated, however, the presence of a well developed scintillation region polewards of the station. Subsequent scintillations at 2106 LT extended more towards the equator. Finally, strong scintillations, extending well towards the equator were recorded at 2311 LT. No scintillations were detected during a morning pass, at 0556 LT. A similar development in the location of scintillations was evident during the second night. Then, the first recorded pass at 2213 LT indicated scintillations towards the pole. The second pass, at 2359 LT, indicated the presence of two scintillation regions towards both the equator and the pole with respect to the station. Scintillation activity was limited to regions between the station and the equator during subsequent passes at 0303 and 0436 LT. No scintillations were detected during a pass at 0450 LT.

The development of scintillation activity tended to follow the pattern shown in Fig. 1. Thus, scintillations during the evening occurred between the station and the pole, at high zenith angles. Later, but still before midnight, the scintil-

Table 1 Number of nights during which displacements in the location of scintillations were observed

	Time (150° EST) of scintillations	
	1800 - 2400	0000 - 5000
Displacement towards equator	22	4
Displacement towards pole	2	1

lation regions split: one remained towards the pole, at somewhat lower zenith angles and the other region shifted towards the equator. Finally, scintillation towards the equator became predominant during the morning hours.

Table 1 shows the number of nights on which displacement of scintillation regions towards the equator occurred. In all but four cases the displacement of scintillations towards the pole, occurred before midnight LT. The number of displacements towards the pole is also shown. It can be seen that scintillations were first recorded between the station and the equator and were noted subsequently polewards of the original position; the reverse trend was infrequent (Table 1).

The presence of scintillations at high zenith angles and polewards of the recording station is a common phenomenon for Australian stations². The spatial effect seems to be strongly dependent on azimuth, and indicates a distinct ridge in scintillation activity. This work shows that the polewards ridge structure may drift towards the equator during the evening hours. A similar scintillation drift was found in the northern hemisphere by Kersley *et al.*⁶ who reported that F-region scintillations moved towards the equator between 1500 and 0200 LT.

It is likely that the scintillations near the pole are associated with field-aligned irregularities at higher latitudes. These irregularities cause pronounced scintillation effects once they have drifted to the position at which the aspect angle is small.

This work was supported financially by the Australian Research Grants Committee.

L. A. HAJKOWICZ

Department of Physics,
University of Queensland, St Lucia,
4067, Australia

Received April 9; revised June 24, 1974.

¹ Jones, K. L., *Planet. Space Sci.*, **17**, 585 (1969).

² Singleton, D. G., *J. atmos. terr. Phys.*, **32**, 789 (1970).

³ Hajkowicz, L. A., *Can. J. Phys.*, **50**, 2654 (1972).

⁴ Hajkowicz, L. A., *Nature phys. Sci.*, **238**, 132 (1972).

⁵ Hajkowicz, L. A., *Planet. Space Sci.*, **22**, 617 (1974).

⁶ Kersley, L., Jenkins, D. B., and Edwards, K. J., *Nature phys. Sci.*, **239**, 11 (1972).

A modified predrift fit of Greenland and western Europe

THE most commonly used predrift fit of north-western Europe against Greenland is based on a least squares fit of the Continental crustal edges which are assumed to follow the 500 fathom submarine contour¹. Recent geophysical studies in the North Atlantic and the Norwegian Sea have established a number of facts bearing directly on the Palaeocene palaeogeography of these areas, and on departures of the continental crustal edge from the 500 fathom contour.

The whole of the Rockall-Hatton Plateau is a submerged continental fragment². Subsidence of this area commenced in the late Palaeocene (56 Myr BP) and the basement dropped to 600–700 m below sealevel by early Eocene times³ (51 Myr BP). The topographic expression of the western edge of the plateau is closely coincident with anomaly 24 (60 Myr BP) indicating that rifting between Greenland and Rockall began at this time.

The Rockall Trough came into existence before late Senonian times, that is, before anomaly 32 (78 Myr BP), (ref. 3). Whether the Rockall Trough owes its origin to seafloor spreading or to subsidence of a continental block

remains an unresolved question but it is now certain that it was in existence for at least 18 Myr before any spreading on the Reykjanes Ridge, and probably for a great deal longer.

There is a gap of approximately 100 km between the continental slope of Greenland (marked by the 1,000 m contour) and the western limit of the oceanic crust (marked by anomaly 24)³ in the area south of latitude 55°N. This may be interpreted in three ways: either the intervening area is one of subsidised continental crust; or there is older oceanic crust between anomaly 24 and the continental slope and therefore an extinct spreading axis which is not in evidence elsewhere in the North Atlantic; or this area was produced by some other, unknown process. The first of these hypotheses seems the most conservative

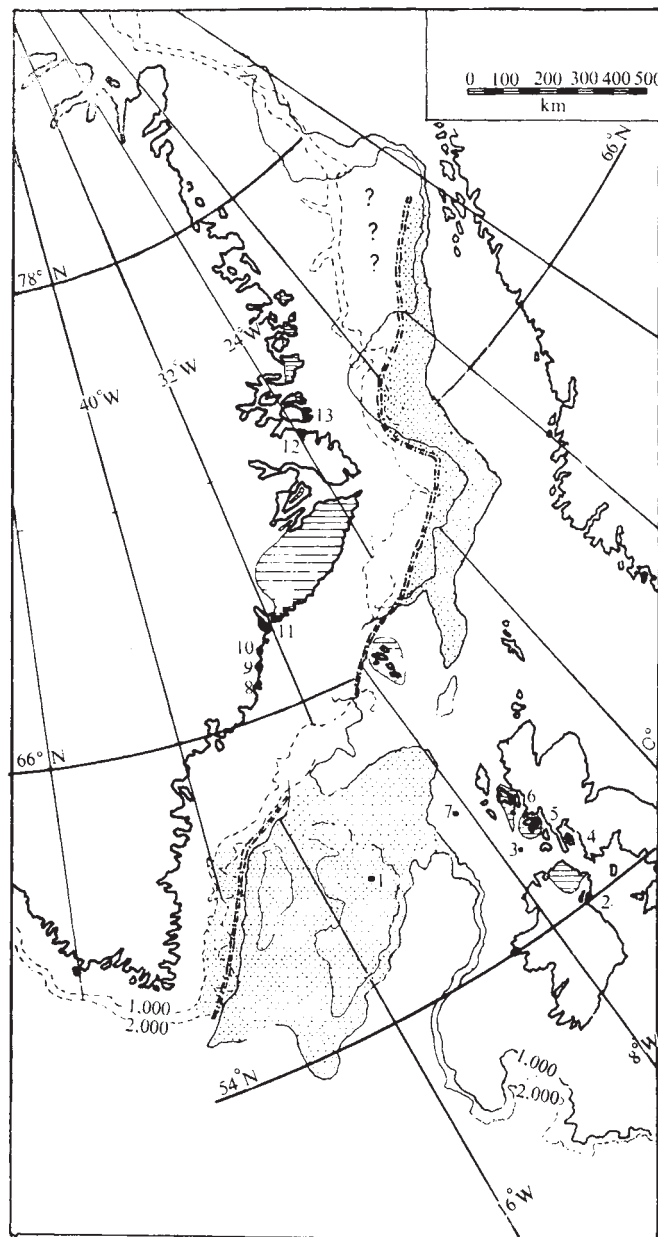


Fig. 1 Predrift reconstruction of Greenland and Europe. Stippled areas, subsidised continental crust; ruled areas, tertiary flood basalts; ---, 1,000 and 2,000 m bathymetric contours around Greenland; —, 1,000 and 2,000 m bathymetric contours around Europe; = =, geophysically defined continental margin. Tertiary plutonic bodies and ring complexes are numbered: 1, Rockall; 2, Mourn; 3, Blackstones Bank; 4, Arran; 5, Mull; 6, Skye; 7, St. Kilda; 8, Cape Gustav Holm; 9, Nualik; 10, Idglutarajik; 11, Skaergaard; 12, Werner Bjerger; 13, Cape Simpson.

in the light of available data from the North Atlantic, and so the continental margin of Greenland is assumed to be coincident with anomaly 24 in this region.

The Faeroe Islands are underlain by continental crust⁴. The junction of the continental crust and the anomalous crust of the Iceland–Faeroe Rise lies approximately 50 km north-west of the Faeroes and is marked by a large gravity anomaly^{5,8}.

The magnetic 'Quiet Zone' of the eastern Norwegian Sea is underlain by deeply subsided continental crust^{7,8}. Between 64°N and 68°N the continental edge is well defined by gravity highs immediately to the seaward side, which are coincident with the limit of magnetic strip anomalies in the area. South of this region, however, the edge is less well defined and is now considered to be at the limit of definable strip anomalies.

Using available information on the geophysically defined continental edge, I have attempted another fit (Fig. 1). This is a subjective reconstruction based on the criterion that areas of known or suspected continental crust should not overlap. The only area in which the margins of the continental masses on opposite sides of the Atlantic are well defined is in the region south of Iceland where anomaly 24 is composed of linear features³. When these predrift isochrons are superimposed the model has only one degree of freedom—relative movement is restricted to a direction parallel to the linear features. Further north, the Jan Mayen and Greenland Fracture Zones apply a restraint to this movement, because the fit must bring into juxtaposition those portions of the features closest to the continents. Thus, the obtained fit has geological rather than statistical significance. In this case the lack of reliable data concerning the position of the continental margin of Greenland north of the Iceland–Greenland Ridge means that objective geometric methods cannot be used to obtain a fit. In this context the large gap created by this reconstruction north of latitude 68°N is not considered as a major obstacle, because it may imply that the continental margin of Greenland in this area has subsided in a manner similar to its Norwegian counterpart.

The new fit differs significantly from that of Bullard *et al.*¹ in that it involves a shift of Greenland some 400 km to the south-west. This brings the Tertiary volcanic province of eastern Greenland into close juxtaposition with the Faeroe Islands and on to the extension of the trend of the Faeroe–Hebridean igneous province, to which it shows both temporal and petrological links. This close spatial relationship supports the suggestion implicit in Holmes⁹ original definition of the Brito–Arctic Province: the rocks are derived from a common source not directly related to other volcanism along the North Atlantic Ridge system. If Iceland is the surface expression of a mantle plume or 'hot spot'^{10–12} which has maintained a fixed position on the Mid-Atlantic Ridge throughout the last 60 Myr (ref. 13) then the traces of the Iceland–Faeroe and Iceland–Greenland Aseismic Ridges should define the relative movement of the continental masses, and any predrift fit constructed on this basis would, necessarily, place the Faeroes opposite the Kangerdlugssuag region of Greenland.

Mesozoic stratigraphy and tectonic patterns in eastern Greenland¹⁴ and north-western Europe show remarkable similarities¹⁵, and the trend of the North Sea Graben, well defined by the major petroleum finds of the area, is coincident with the trend of the Mesozoic graben structure of eastern Greenland (Fig. 2). Although the thickness¹⁸ of sediment in the North Sea (up to 8 km) is considerably greater than in Greenland, much of this is of Tertiary age and the thickness of Mesozoic sediment (approximately 4 km) is more closely comparable to that of Greenland. If these structures form a truly continuous belt then they cut across the subsequent rift system of the North Atlantic, and the view that the Mesozoic and Tertiary basins of

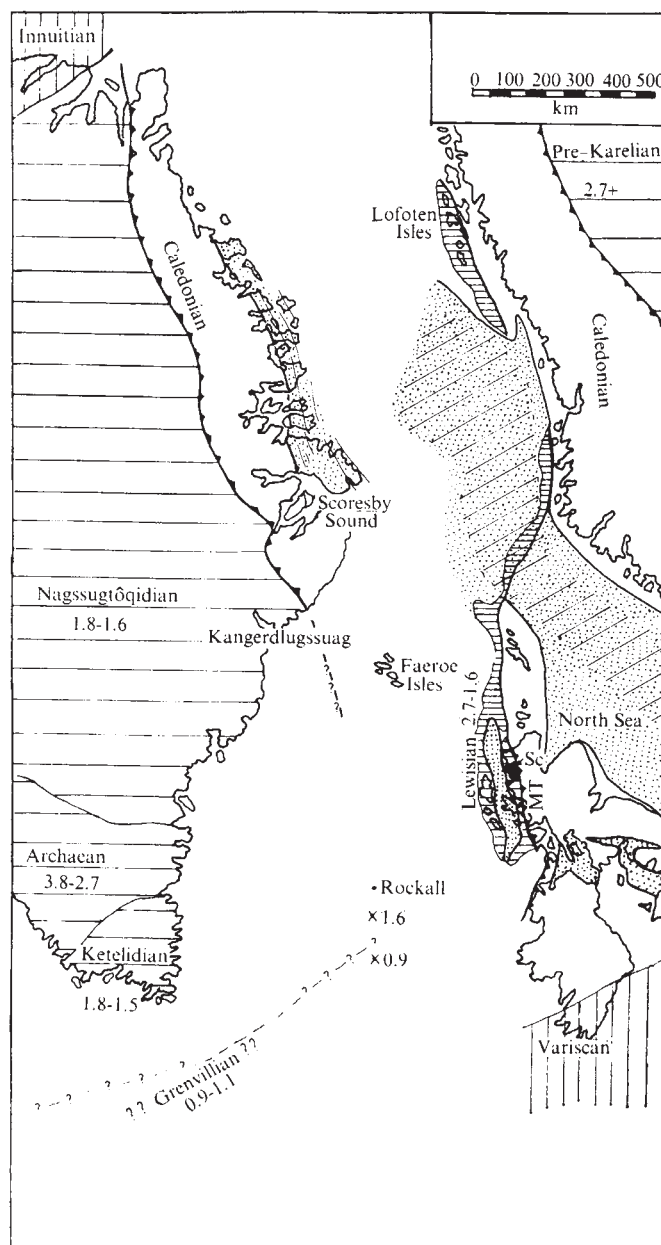


Fig. 2 Major geological features of Greenland and north-western Europe. Horizontal ruling, pre-Caledonian orogenic belts showing radiometric ages ($\times 10^9$ yr); vertical ruling, post-Caledonian orogenic belts; stipple, Mesozoic basins; Sc, Scourian; MT, Moine Thrust; x, drill sites on the Rockall Plateau, with radiometric ages ($\times 10^9$ yr).

north-western Europe are, in a simple way, related to this incipient rift must be examined more closely^{17–19}.

The difficulty of aligning the Caledonian Front of Greenland¹⁴ with the Moine Thrust Zone of north-western Scotland has been cited as criticism of the Bullard fit²⁰. But this was overcome to a large extent, by Bott and Watts²¹, whose reconstruction produced a reasonable alignment of these features. The present model, however, is not compatible with the alignment of these two structures (Fig. 2). An examination of the geophysical evidence concerning the north-eastward extension of the Precambrian basement of north-western Scotland^{22,23,8} strongly suggests that it takes the form of a discontinuous basement ridge trending NNE and eventually reappearing in the Lofoten Islands. The basement complex of the Lofoten Islands has a radiometric history similar to that of the Laxfordian of Scotland^{24,25}. The published ages of rocks drilled from the Rockall Plateau^{26,27} do little to clarify the position of the

southerly continuation of the Caledonian Front from Greenland. The date of 989 ± 5 Myr from one of the samples (Fig. 2), assumed to be Grenvillian, is, however, similar to dates from immediately south-west of the Caledonian Front in Greenland²⁸.

The suggestion that the Precambrian histories of Scotland and Greenland are directly comparable and that the Scourian is the lateral equivalent of the Archaean of Greenland²⁹ requires further corroboration in view of the fact that these two regions must, on the reconstruction presented here, be assumed to be separated by the western half of the Caledonian Mountain Belt.

I acknowledge discussions with Drs D. R. Bowes, J. Hall, A. C. McLean and D. W. Powell.

IAN R. VANN

Department of Geology,
The University,
Glasgow G12 8QQ, UK

Received April 23; revised June 18, 1974.

- ¹ Bullard, E. C., Everett, J. E., and Smith, A. G., *Phil. Trans. R. Soc.*, **A258**, 41–51 (1965).
- ² Scrutton, R. A., and Roberts, D. G., *Rep. Inst. geol. Sci.*, 70/14, 77–87 (1971).
- ³ Laughton, A. S., et al., *Init. Rep. Deep Sea Drilling Project, Volume XII* (U.S. Government Printing Office, Washington, 1972).
- ⁴ Bott, M. H. P., Sunderland, J., Smith, P. J., Casten, U., and Saxov, S., *Nature*, **248**, 202–204, (1974).
- ⁵ Bott, M. H. P., Browitt, C. W. A., and Stacey, A. P., *Mar. geophys. Res.*, **1**, 328–342 (1971).
- ⁶ Bott, M. H. P., *Implications of Continental Drift to the Earth Sciences* (edit. by Tarling, D. H., and Runcorn, S. K.), **1**, 175 (Academic Press, London, 1973).
- ⁷ Talwani, M., and Eldholm, O., *Nature*, **241**, 325–330 (1973).
- ⁸ Talwani, M., and Eldholm, O., *Bull. geol. Soc. Am.*, **83**, 3575–3606 (1973).
- ⁹ Holmes, A., *Mineralog. Mag.*, **18**, 180–223 (1918).
- ¹⁰ Morgan, W., *Nature*, **239**, 42–43 (1971).
- ¹¹ Vogt, P. R., *Nature*, **240**, 338–342 (1972).
- ¹² Schilling, J. G., *Nature*, **242**, 565–571 (1973).
- ¹³ Minister, J. B., Jordan, T. H., and Molnar, P., *Geophys. J. R. astr. Soc.*, **36**, 541–576 (1974).
- ¹⁴ Haller, J., *Geology of the East Greenland Caledonides* (Wiley Interscience, London, 1971).
- ¹⁵ Hallam, A., *J. Geol.*, **79**, 129–157 (1971).
- ¹⁶ Kent, P. E., *Proc. Yorks. geol. Soc.*, **36**, 1–22 (1967).
- ¹⁷ Bott, M. H. P., *Tectonophysics*, **11**, 319–327 (1971).
- ¹⁸ Hallam, A., *Earth planet. Sci. Lett.*, **16**, 171–177 (1972).
- ¹⁹ Hall, J., and Smythe, D. K., *Earth planet. Sci. Lett.*, **19**, 54–59 (1973).
- ²⁰ Harland, W. B., *Phil. Trans. R. Soc.*, **A258**, 59–75 (1965).
- ²¹ Bott, M. H. B., and Watts, A. B., *Rep. Inst. geol. Sci.*, 70/14, 93–109 (1971).
- ²² Eden, R. A., Wright, J. E., and Bullerwell, W., *Rep. Inst. geol. Sci.*, 70/14, 115–128 (1971).
- ²³ Watts, A. B., *Scott. J. Geol.*, **7**, 189–218 (1971).
- ²⁴ Heier, K. S., and Compston, W., *Norsk. geol. Tidsskr.*, **49**, 257–283 (1969).
- ²⁵ Bowes, D. R., *Mem. Am. Ass. Petrol. Geol.*, **12**, 575–594 (1969).
- ²⁶ Miller, J. A., Mathews, D. H., and Roberts, D. G., *Nature phys. Sci.*, **246**, 61 (1973).
- ²⁷ Roberts, D. G., Ardus, D. A., and Dearnley, R., *Nature phys. Sci.*, **244**, 21 (1973).
- ²⁸ Hansen, B. T., Oberli, F., and Steiger, R. H., *Third European Colloquium of Geochronology*, Abstr. No. 81 (Oxford, 1973).
- ²⁹ Bridgewater, D., Watson, J., and Windley, B. F., *Phil. Trans. R. Soc.*, **A273**, 493–512 (1973).

venance. They concluded that “any suggestion that the Burtle Beds are ice transported is quite untenable. A fluvioglacial origin is . . . so unlikely as to be unacceptable. . .”.

As evidence for a marine origin Kidson and Haynes showed that the microfauna indicate a transgressive estuarine-marine phase. The sands and gravels rest conformably on clays of similar age and origin which were clearly laid down in the same transgression. The clays in turn rest on a hard fissile clay interpreted as Lower Lias. Hawkins and Kellaway¹, in reply, found “nothing to convince us of the marine origin of the Burtle Beds or to change our opinions of the older glaciations”. They suggested that the clay beneath the sand described by Kidson and Haynes was “remoulded Lower Lias clay contaminated with microfossils or ground up shell debris,

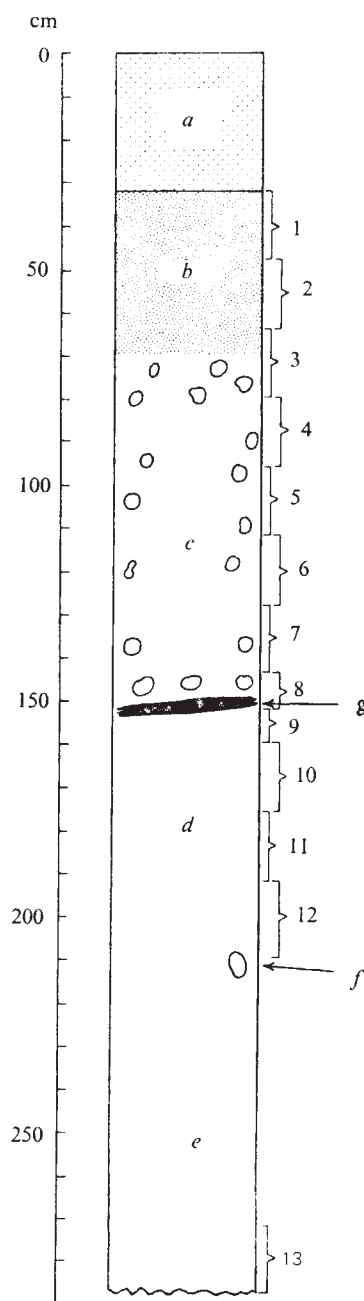


Fig. 1 The section exposed in a pit at Hill Farm, Catcott Burtle. a, Sandy soil; b, sand; c, brown clay with stones and occasional shells; d, hard fissile blue-yellow clay; e, hard fissile blue clay; f, *Palaeochoceras elicium* (ammonite); g, *Corylus* (recumbent branch?). Numbers refer to samples (see Fig. 2).

The Burtle Beds of Somerset—glacial or marine?

KELLAWAY¹ described the Burtle Beds as “residual masses of glacial sand and gravel (‘Burtle Beds’), (which) rise out of the marshes at Catcott Burtle, Weston Zoyland and Othry”. Kidson and Haynes² supported the earlier view of Bulleid and Jackson³ that the deposits are of marine origin and of interglacial rather than glacial age and pro-

Fauna		Samples						
		1	3	5	7	9	11	13
Quaternary microfauna	Foraminifera	AMMONIA LIMNETES						
		A. SPECIES		■	■	■		
		BULIMINA ELONGATA						
		B. GIBBA		●				
		CIBICIDES LOBATULUS		■	■	■		
		ELPHIDIUM cf. ADVENUM		■	■	■		
		E. CLAVATUM		■	■	■		
		E. CRISPUM		■	■	■		
		E. FITCHTELIANUM		■	■	■		
		E. MARGARITACEUM		■	■	■		
Quaternary microfauna	Ostracods	E. SELSEYENSE		○	○	○		
		E. SPECIES		○	○	○		
		E. WADDENSIS		○	○	○		
		E. WILLIAMSONI		○	○	○		
		FISSURINA ORBIGNYANA		○	○	○		
		GYPSSINA VESICULARIS		○	○	○		
		LENTICULINA SUBORBICULARIS		○	○	○		
		PROTOLPHIDIUM ANGLICUM		○	○	○		
		ROSALINA WILLIAMSONI		○	○	○		
		R. MILLETI		○	○	○		
Lias microfauna (derived)	Foraminifera	TRIFARINA ANGULOSA		○	○	○		
		AURILA CONVEXA		○	○	○		
		CALLISTOCYHERE AMARA		○	○	○		
		CYPRIDEIS TOROSA		○	○	○		
		CYTHOPELON NODOSUM		○	○	○		
		HEMICYHERE CLATHRATA		○	○	○		
		H. VILLOSA		○	○	○		
		LEPTOCYHERE CONFUSA		○	○	○		
		L. LACERTOSA		○	○	○		
		L. PELLUCIDA		○	○	○		
Lias microfauna (derived)	Ostracods	LOXOCOCHA ELLIPTICA		○	○	○		
		NEREINA ANGULATA		○	○	○		
		UROCYTHEREIS DISTINGUENDA		○	○	○		
		AMMODISCUS ASPER		○	○	○		
		ASTACOLUS GOTTINGENSIS		○	○	○		
		A. cf. PAUPERATUS		○	○	○		
		A. cf. PRIMUS		○	○	○		
		CITHARINA INAEQUISTRATA		○	○	○		
		C. SPECIES		○	○	○		
		DENTALINA MATUTINA		○	○	○		
Lias microfauna (derived)	Foraminifera	D. cf. TERQUEMI		○	○	○		
		D. SPECIES		○	○	○		
		FRONCULARIA BICOSTATA		○	○	○		
		HAPLOPHRAGMOIDES SPECIES		○	○	○		
		INVOLUTINA LIASSICA		○	○	○		
		LAGENA OVATA		○	○	○		
		LENTICULINA VARIANS		○	○	○		
		LINGULINA TENERA		○	○	○		
		MARGINULINA PRIMA		○	○	○		
		NODOSARIA cf. METTENSIS		○	○	○		
Lias microfauna (derived)	Ostracods	N. PRIMITIVA		○	○	○		
		N. SPECIES		○	○	○		
		SPIRILLINA INFIMA		○	○	○		
		TROCHAMMINA SPECIES		○	○	○		
		BAIRDIA MOLESTA		○	○	○		
		CYTHARELLA SPECIES		○	○	○		
		KRAUSSELLA? LANCEOLATA		○	○	○		
		OGMOCONCHA ELLIPSOIDEA		○	○	○		
		O. HAGENOWI		○	○	○		
		PROCYTHERIDEA SPECIES		○	○	○		
Lias microfauna (derived)	Miscellanea	BRYOZOAN FRAGMENTS		○	○	○		
		ECHINOID SPINES		○	○	○		
		WORM TUBES		○	○	○		
		GASTEROPOD CASTS		○	○	○		
		PENTACRINUS OSSICLES		○	○	○		
		BIVALVE CASTS		○	○	○		
		BRACHIOPOD CASTS		○	○	○		
		Symbols showing numbers		○	○	○		
		in 50gms. start weight		○	○	○		
				○	○	○		

Fig. 2 The distribution of fauna in the samples from Hill Farm, Catcott.

some having been carried by water draining from the overlying Burtle Beds in the boreholes".

To test this argument, we dug a pit with a mechanical excavator so that samples could be obtained from a clean face where any possibility of contamination could be ruled out. The pit was sited midway between boreholes 8D and 8E (Fig. 2 of ref. 2) at Hill Farm, Catcott Burtle (ST399430). The stratigraphy revealed is shown in Fig. 1. At the passage between the two clays was a branch, root or trunk of *Corylus* (hazel), not in a growth position, which yielded a ^{14}C date of $4,280 \pm 70$ b.p. (HAR 463). The face was sampled as indicated in Fig. 1, and alternate samples were examined for microfauna (Fig. 2). The results clearly confirm previous observations²: first, that the microfauna

in the Burtle clay (the upper brown clay) is a mixture of Quaternary and Liassic species with other time periods apparently unrepresented; second that there are no exclusively cold water Quaternary species; and finally that the overall aspect is consistent with estuarine accumulation. The samples were also examined for pollen, but it has proved impossible to date this or other 'Burtle Bed' deposits by pollen analysis.

When the distribution chart is considered in detail and compared with that for the boreholes previously examined (Fig. 3 of ref. 2) the close similarity of the microfaunas, particularly with 8D, is apparent, allowing for name changes in some of the ostracods, because of generic shifts^{5,6}.

The deposit from which the name 'Burtle Beds' was derived is thus clearly seen at Catcott Burtle to be of estuarine-marine origin and to include Quaternary clays beneath the sands and gravels, as suggested earlier².

A completely new element has appeared, however, because of the discovery of *Corylus* in the pit at Hill Farm. The wood occurs immediately above the passage horizon represented by sample 9 (Fig. 1). If the Burtle clays were of Pleistocene age, an infinite ^{14}C age could be expected. The Holocene date can only mean, however, that the overlying deposits were emplaced in the late Holocene. A sand-intertidal silt relationship, similar to that at Hill Farm, is evident in an excavation for a new road bridge near Penzoy Farm (ST337349), (D. D. Gilbertson, personal communication). A sample of wood from Penzoy Farm is now being dated, but preliminary results suggest a similar Holocene age. An examination of the altitude of the wood samples at Hill Farm and Penzoy Farm (3.5 m OD), which may be either *in situ* or driftwood, in relation to Flandrian sealevel curves for the area⁷ suggests strongly that these sites were coastal towards the end of that transgression. They therefore cannot now be regarded as typical Burtle bed sites. It is possible that these sites are of interglacial age, and were modified in post-glacial times. It is interesting to note, in retrospect, that Ussher⁸ discussed and rejected the possibility of a Flandrian age for the beds.

The division into a sand layer, and an underlying clay bed of similar age, arrived at on the basis of borehole evidence, has been demonstrated in an open face at Hill Farm, Catcott Burtle, eliminating the argument about contamination. A comparable division was revealed in a pit at Greylake. There can be no doubt about the Interglacial age of these deposits. If the sands and gravels at Catcott Burtle were originally of true Burtle Bed type comparable to those at Greylake, they must have been reworked, at least on the margins, by the Flandrian sea. The microfauna includes forms² that are known from the Flandrian and Recent of Great Britain and also occur in the last interglacial (Ipswichian). The Catcott Burtle fauna does, however, include northern forms, *Elphidium clavatum* and *Hemicythere clathrata*, and a southern form *E. fitchtelianum*, which lends slight support to the idea of reworking. On the other hand these deposits may be truly of Flandrian age and unrelated to what are now called Burtle Beds elsewhere in the levels.

These conclusions do not call into question the age or origin of the mass of the Burtle Beds. No evidence has yet been produced on which to justify a glacial or fluvioglacial origin for them. All the available evidence², including the pre-Flandrian shell dates from Mollusca from the Burtle sands and gravels south of the Poldens⁹ points to a marine interglacial, probably Ipswichian, origin. The problem remains of separating the undisturbed deposits of Burtle sands and gravels from the deposits of the Flandrian sea or from earlier marine beds reworked by that sea. That now seems to have washed not only islands and peninsulas of Lias and Trias, but also of Burtle sands and gravels. On the basis of height alone, most of the sand

batches north of the Poldens and perhaps many of those to the south must be re-examined. More detailed study is called for than has yet been attempted. A beginning is being made with a detailed re-examination of the deposits of Greylake.

Dr R. C. Whatley identified the ostracods, and the results were discussed with Dr Alan Lord (University College London), Dr C. G. Adams (British Museum, Natural History) and Mr Ben Johnson (Arco Petroleum Co.) kindly examined and commented on the Liassic foraminiferal fauna.

C. KIDSON

Department of Geography

J. R. HAYNES

Department of Geology

A. HEYWORTH

Department of Geography
University College of Wales,
Penglais, Aberystwyth,
Cardiganshire, UK

Received April 8; revised June 6, 1974.

¹ Kellaway, G. A., *Nature*, **233**, 30 (1971).

² Kidson, C., and Haynes, J. R., *Nature*, **239**, 390 (1972).

³ Bulleid, A., and Jackson, J. W., *Proc. Somerset. archaeol. nat. Hist. Soc.*, **83**, 111 (1941).

⁴ Hawkins, A. B., and Kellaway, G. A., *Nature*, **243**, 216 (1973).

⁵ Lord, A., *Palaeontology*, **14**, 642 (1971).

⁶ Whittaker, J. E., thesis, Univ. Wales (1972).

⁷ Kidson, C., and Heyworth, A., *Proc. Ussher Soc.*, **2**, 565 (1973).

⁸ Ussher, W. J. E., *Proc. Somerset. archaeol. nat. Hist. Soc.*, **60**, 17 (1914).

⁹ Kidson, C., in *Exeter Essays in Geography* (edit. by Gregory, K. J., and Ravenhill, W.), 1 (Exeter, 1971).

Dating of the rock succession containing fossil hominids at East Rudolf, Kenya

THE geology of the large complex faulted depression between the Ethiopian and Kenyan rifted, upwarped domes, is virtually unknown in detail. Up until 1969 there had been only a few isolated expeditions¹⁻⁴ into the region, but since then there has been an increased amount of research around Lake Rudolf, particularly by Kenyan, American and French based workers⁵. It has been shown that the sparsely inhabited semi-desert area to the east of Lake Rudolf is exceptionally rich in the remains of fossil man, and there is abundant evidence of his activities⁵⁻²⁸: informative hominid assemblages have been found there together with possibly the oldest known archaeological traces of hominid activity.

The Plio-Pleistocene rock succession within the East Rudolf sedimentary basin contains several prominent felsic volcanic tuffs. The more extensive of these tuff horizons are vitally important for both stratigraphical subdivision and relationships²⁹, and the dating of fossil faunas within the sequence. At an early stage of the exploratory work at East Rudolf we were able to obtain a date of 2.61 ± 0.26 Myr for the important KBS tuff horizon⁷, using argon-40/argon-39 age spectrum analysis of a collection of large fresh sanidine crystals which had been separated from KBS pumice cobbles in the field. Dating of the other tuff horizons was, however, more difficult.

Certain depositional and other characteristics of the East Rudolf tuffs suggest that the base of each major tuff horizon can be regarded as an isochronous surface within rather narrow limits. Thus, by mapping dated tuffs and their

lateral equivalents and considering information from the geomagnetic reversal stratigraphy²² it should be possible to produce a detailed geochronological map of East Rudolf, from which the age of any faunal or archaeological site can be estimated with close precision.

A complex of Miocene-Recent volcanics and sediments borders the East Rudolf Basin on its entire eastern side. The complex is cut by the main Pleistocene rift which runs between the Sugata Valley and Lake Stephanie. A number of detailed studies within this area will be necessary in order to identify the provenance of the sediments and tuffs in the East Rudolf Basin and to resolve questions about the location of the ancient drainage channels and the character and timing of the source eruptions.

There is wide interest in the fossil hominids from East Rudolf; the cranium KNM-ER 1470 has attracted particular attention. It is, therefore, appropriate to discuss summarily the main conclusion of our 1970-1973 dating programme, although full publication of the results of these and other projects must await the completion of the field mapping.

Over one hundred rock and mineral samples from East Rudolf have been analysed by a combination of either conventional K-Ar, total degassing and spectrum ⁴⁰Ar/³⁹Ar age or fission track dating methods²⁹⁻³⁵. Figure 1 summarises our present conclusions regarding the East Rudolf time sequence, and illustrates the relationship between the dated tuffs and the world wide geomagnetic polarity reversal time scale. The relationship of hominid fossil sites to the dated tuffs has been summarised by Brock and Isaac²².

Dating work on a sanidine concentrate from a single pumic lump taken from the Chari Tuff, at Ileret (Fig. 1)

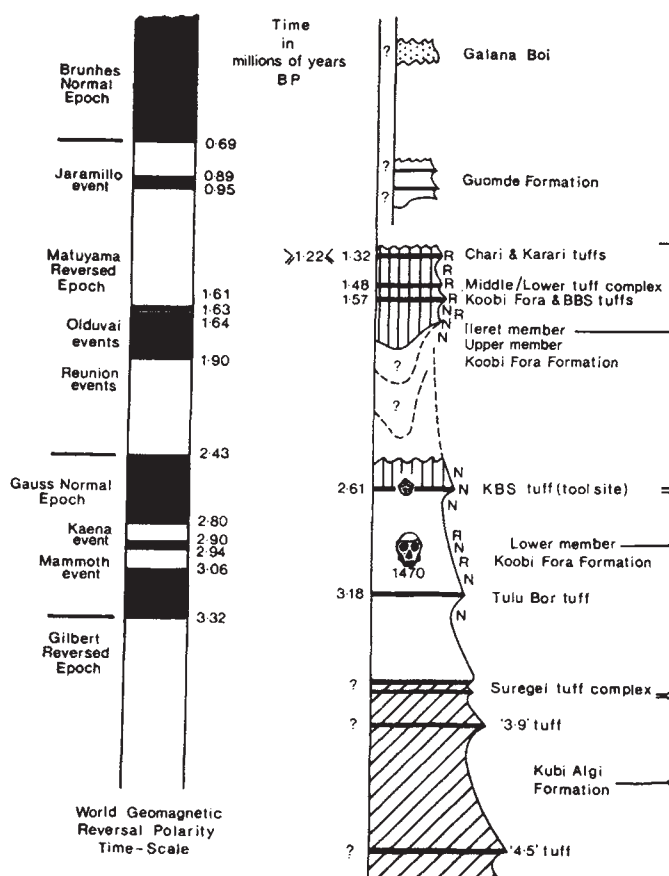


Fig. 1 Dated tuffs in the East Rudolf sedimentary basin. Palaeomagnetic data from Brock and Isaac²² and stratigraphical nomenclature from Bowen and Vondra³⁰.

suggested minimum apparent ages of 1.2 Myr from total degassing analysis, and 1.28 ± 0.23 Myr from age spectrum analysis. Age spectrum analysis of another sanidine concentrate revealed minor Ar loss and excess Ar contamination effects, and indicated a minimum apparent age of 1.22 ± 0.01 Myr for the major juvenile sanidine component.

An apparent age of 1.48 ± 0.17 Myr was obtained by total degassing analysis of sanidine concentrates from pumice taken from the Lower-Middle Tuff Complex, at Ileret (Fig. 1).

Apparent ages obtained by total degassing analysis from seven independent sanidine concentrates from the Karari Tuff (Fig. 1) ranged from 0.6 to 1.39 Myr. Age spectrum analysis revealed that both argon loss and excess argon errors are present in these concentrates. A close maximum apparent age of 1.32 ± 0.10 Myr was obtained for the juvenile sanidine component by age spectrum analysis.

During age measurements of the Koobi Fora Tuff (Fig. 1) it was difficult to avoid the effects of partial and complete overprinting by later events, and the effect of contamination by xenolithic and/or detrital material. Imprecise age spectrum results at first suggested an apparent age of 1.1 ± 1.7 Myr, whereas total degassing apparent ages, obtained from a large number of independent samples, ranged from 1.53 to 1.69 Myr. Further age spectrum studies indicated Ar loss and excess Ar errors, a minimum apparent age of 1.48 ± 0.23 Myr, and a close apparent age of 1.57 ± 0.00 Myr for the major juvenile sanidine component. Evidence of severe overprinting at around 1.0 Myr was also obtained.

Apparent ages ranging from 0.87 to 1.66 Myr were obtained from the BBS Tuff Complex (Fig. 1) by total degassing analysis of seven independent sanidine concentrates from pumice. Age spectrum analysis revealed both Ar loss and contamination errors. The two principal feldspar components in the sample analysed using this method, have apparent ages of 1.70 ± 0.04 Myr and 1.56 ± 0.02 Myr, respectively.

A considerable amount of dating work has been done on samples from the KBS tuff (Fig. 1) Although the sanidine component in this tuff has certainly been subjected to a number of partial or complete overprinting events, severe contamination or excess argon effects have not been identified. Total degassing apparent ages from ten independent sanidine concentrates range between 0.52 and 2.64 Myr. Seven age spectrum analyses of further, independent KBS sanidine concentrates identified two age components: crystallisation at an apparent age of 2.61 ± 0.26 Myr with a minimum apparent age of 2.54 ± 0.53 Myr; and overprinting by later events at about 2.42 ± 0.02 Myr, 2.01 ± 0.03 Myr, 1.75 Myr and 1.0 Myr (all apparent ages).

Age spectrum analysis of a sanidine concentrate from Tulu Bor pumice (Fig. 1) revealed it to be a mixture of accessory sanidine with an apparent age of 4.0 ± 0.01 Myr, and juvenile sanidine with an apparent age of 3.18 ± 0.09 Myr.

Two tuffs in the Kubi Algi Formation (Fig. 1) were examined using a number of dating methods. So far, however, only very imprecise indications of apparent age have been obtained. These are about 3.9 and 4.5 Myr, respectively.

Plio-Pleistocene plateau basalts capping the volcanic terrain north-east of the sedimentary basin have an average K-Ar minimum apparent age of 2.20 ± 0.04 Myr. The Kokoi basalts occurring within the basin have been dated using K-Ar methods, at an average apparent age of 3.62 ± 0.36 Myr. Similar basalts entering the basin from the south-east near Kubi Algi have average apparent K-Ar ages of 3.8 ± 0.4 Myr and 3.80 ± 0.38 Myr.

Miocene peralkaline rhyolites at Kubi Algi have average K-Ar apparent ages of 7.5 ± 0.8 Myr. An obsidian from Shin has been dated using the K-Ar method, at an apparent age of 11.8 ± 0.9 Myr. A variety of basalts exposed in the

area of the Suregei Cuesta have average K-Ar apparent ages between 11.6 ± 0.5 Myr and 14.1 ± 1.4 Myr. Several extensive eutaxitic felsic ignimbrites found in the Ethiopia-Kenya border area, near Gum Dura, have average K-Ar apparent ages near to 16.15 ± 0.12 Myr. The oldest basalts which have been dated, have average K-Ar apparent ages around 17.3 ± 1.11 Myr, and outcrop in the Buluk Gap and near Fora water hole.

This dating work on rocks from the East Rudolf Basin, completed in 1973, confirms the suggested age of 2.61 ± 0.26 Myr reported for the KBS tuff in 1970 (ref. 7), and places it securely within a chronologically satisfactory sequence of dated tuffs. Our conclusions are compatible with palaeomagnetic polarity reversal results from sediments and tuffs in the basin²². The compatibility of independent evidence is a very strong argument for accepting the chronology now proposed for East Rudolf. A discrepancy between this chronology and the middle part of that proposed by Brown and Lajoie³⁶ for the Shungura Formation of the Omo Valley of southern Ethiopia is suggested by detailed faunal correlation between the two areas³⁷. This is under joint investigation. Important episodes of hydrothermal overprinting affected the rocks of the East Rudolf Basin between 2.05 Myr and 1.75 Myr age and again at about 1.1 Myr and 0.5 Myr age. We believe that these overprints have been caused by thermal activity related to magmatic doming, major rift movements and concomitant volcanism. It is significant that the three major rift faulting events in Kenya and Tanzania have been dated at 2.1 Myr, 1.2 Myr and near to 0.4 Myr, respectively³⁸.

This work has received support and encouragement from R. E. F. Leakey, G. Li. Isaac, K. Kimeu, members and field assistants of the East Rudolf Research Project, the National Museums of Kenya, the Royal Society, the Natural Environment Research Council, and the Universities of London and Cambridge.

FRANK J. FITCH
JAN C. FINDLATER
RONALD T. WATKINS

Department of Geology,
Birkbeck College,
University of London,
7/15 Gresse Street, London W1P 1PA, UK

J. A. MILLER
Department of Geodesy and Geophysics,
University of Cambridge,
Madingley Rise,
Cambridge, UK

Received November 21, 1973.

- ¹ Fuchs, V. E., *Phil. Trans. R. Soc.*, **B229**, 219 (1939).
- ² Mohr, P. A., *The Geology of Ethiopia* (Univ. College Addis Ababa Press, Addis Ababa, 1964).
- ³ Walsh, J., and Dodson, R. G., *Rep. geol. Surv. Kenya*, **82**, (1969).
- ⁴ Butzer, K. W., *Recent History of an Ethiopian Delta* (Univ. Chicago Department of Geography Research Paper No. 136, 1971).
- ⁵ Leakey, R. E. F., *Nature*, **226**, 223 (1970).
- ⁶ Behrensmeyer, A. K., *Nature*, **226**, 225 (1970).
- ⁷ Fitch, F. J., and Miller, J. A., *Nature*, **226**, 226 (1970).
- ⁸ Leakey, M. D., *Nature*, **226**, 228 (1970).
- ⁹ Leakey, R. E. F., *Nature*, **231**, 241 (1971).
- ¹⁰ Vondra, C. F., Johnson, G. D., Bowen, B. E., and Behrensmeyer, A. K., *Nature*, **231**, 245 (1971).
- ¹¹ Maglio, V. E., *Nature*, **231**, 248 (1971).
- ¹² Leakey, R. E. F., Mungai, J. M., and Walker, A. C., *Am. J. phys. Anthropol.*, **35**, 175 (1971).
- ¹³ Isaac, G. L., Leakey, R. E. F., and Behrensmeyer, A. K., *Science*, **173**, 1129 (1971).
- ¹⁴ Leakey, R. E. F., *Nature*, **237**, 264 (1972).
- ¹⁵ Maglio, V. E., *Nature*, **239**, 379 (1972).
- ¹⁶ Leakey, R. E. F., Mungai, J. M., and Walker, A. C., *Am. J. phys. Anthropol.*, **36**, 235 (1972).
- ¹⁷ Cooke, H. B. S., and Maglio, V. E., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J. A.), (Scottish Academic Press, Edinburgh, 1972).

- ¹⁸ Leakey, R. E. F., *Soc. Biol.*, **19**, 99 (1972).
- ¹⁹ Leakey, R. E. F., *Nature*, **242**, 170 (1973).
- ²⁰ Bowen, B. E., and Vondra, C. F., *Nature*, **242**, 391 (1973).
- ²¹ Leakey, R. E. F., *Nature*, **242**, 447 (1973).
- ²² Brock, A., and Isaac, G. L., *Nature*, **247**, 344 (1974).
- ²³ Day, M. H., and Leakey, R. E. F., *Am. J. phys. Anthropol.* (in the press).
- ²⁴ Day, M. H., and Leakey, R. E. F., *Am. J. phys. Anthropol.* (in the press).
- ²⁵ Leakey, M. G., and Leakey, R. E. F., *Fossil Vertebrates of Africa*, vol. III (in the press).
- ²⁶ Leakey, R. E. F., and Walker, A. C., *Am. J. phys. Anthropol.* (in the press).
- ²⁷ Leakey, R. E. F., and Wood, B. A., *Am. J. phys. Anthropol.* (in the press).
- ²⁸ Walker, A. C., *J. hum. Evol.* (in the press).
- ²⁹ Miller, J. A., and Fitch, F. J., in *The Phanerozoic Time scale* (edit. by Harland, W. B., et al.), (Geol. Soc. Lond., London, 1964).
- ³⁰ Fitch, F. J., Miller, J. A., and Mitchell, J. G., in *Time and Place in Orogeny* (edit. by Kent, P. E., et al.), (Geol. Soc. Lond., London, 1969).
- ³¹ Fitch, F. J., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J. A.), (Scottish Academic Press, Edinburgh, 1972); Miller, J. A., *ibid.*
- ³² Fitch, F. J., and Miller, J. A., *J. geol. Soc. Lond.*, **127**, 277 (1971).
- ³³ Fitch, F. J., and Miller, J. A., in *Symposium on Granites, Gneisses and related Rocks (Granite '71)*, (Geol. Soc. Un. S.Afr., Pretoria, in the press).
- ³⁴ Fitch, F. J., Forster, S. C., and Miller, J. A., *Rep. Prog. Phys.* (in the press).
- ³⁵ Hurford, A. J., *Nature*, **249**, 236 (1974).
- ³⁶ Brown, F. H., and Lajoie, K. R., *Nature*, **229**, 483 (1971).
- ³⁷ *Stratigraphy, Palaeoecology and Evolution in the Lake Rudolf Basin* (edit. by Coppens, Y., Isaac, G. L., and Howell, F. C.), (Univ. Chicago Press, Chicago, in the press).
- ³⁸ Macintyre, R. M., Mitchell, J. G., and Dawson, J. B., *Nature*, **247**, 354 (1974).

Probing broad lines in molecular spectra by optical site selection spectroscopy

WE report evidence that suggests that narrow-line spectra can be obtained for many organic compounds without the difficulties commonly found with traditional mixed-crystal line-narrowing techniques. Furthermore, our findings suggest a new method by which low temperature line-broadening mechanisms may be studied.

Even at very low temperatures the optical absorption and fluorescence bandwidths of many organic compounds in solution remain very broad, typically several hundred cm^{-1} , with unresolved or poorly resolved structure. The techniques of mixed crystal spectroscopy^{1,2} and its more recent variant due to Shpol'skii³ have been developed to probe whatever structure may be hidden in these broad lines or bands. (The spectra of neat crystals often yield sharp lines at helium temperatures but these spectra are often more representative of collective excitations or excitons and not the 'unperturbed' molecular spectrum.) Unfortunately, narrow-line mixed-crystal and Shpol'skii spectra can be obtained only in those special instances in which a suitable host crystal or quasi-crystal can be made to fit the subject molecule. The purpose of the host is to hold every subject molecule in the sample in one or another of a small number of available environments (crystal fields). For most organic compounds suitable hosts are not known.

The method for obtaining narrow-line spectra which we describe here was first demonstrated by Szabo⁴, in a study of ruby. We conclude from studies of a variety of organic compounds that the method is indeed useful in obtaining high resolution spectra of organic molecules in low temperature solutions. It seems to be much more flexible than the mixed-crystal and Shpol'skii methods because it discards the very special host matrices on which those tech-

niques are based and can be used with a variety of hosts including glasses and polymers.

It seems probable that the vibronic lines of two identical molecules in a frozen amorphous host solution are shifted with respect to each other if and when their local environments differ, and that in many host media the shift may far exceed the widths of the individual vibronic lines. Furthermore, it is a natural assumption that a molecule interacts optically only with light that is resonant with one of its vibronic lines.

If these assertions are approximately true, then it should be possible to do 'mixed-crystal' spectroscopy in arbitrary vitreous hosts simply by using optical probes that are spectrally very narrow, because the light itself will pick out only those subject molecules that happen to be in identical local environments or sites in the host. For convenience we will term this technique 'optical site selection spectroscopy'.

We have observed, from a variety of organic compounds, narrow-line fluorescence similar to that reported by Persson et al., for perylene⁵. In every case we used spectrally

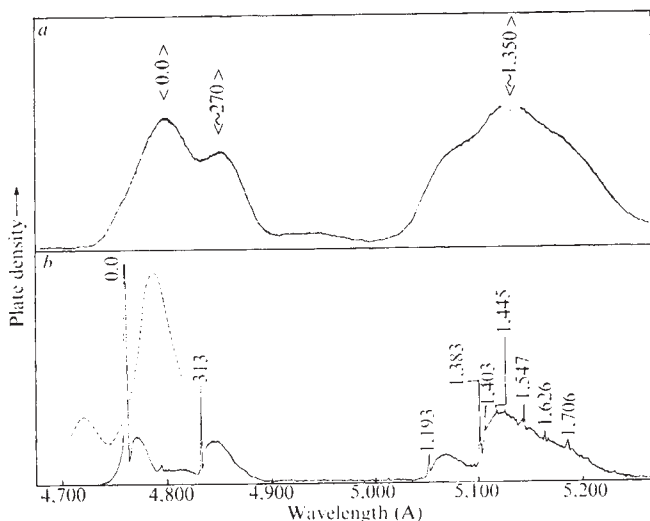


Fig. 1 The fluorescence spectrum of a 10^{-4} M solution of naphthalene in a 2-methyltetrahydrofuran glass at 4.2 K: *a*, Excited with broadband (~ 100 Å) Hg line at 4,358 Å; *b*, excited with 4,765 Å argon ion laser line (bandwidth < 0.2 cm^{-1}). Dotted curve is a portion of the absorption spectrum of naphthalene at 4.2 K. The 0.0 bands are taken as the origins. The distances to succeeding peaks are given in cm^{-1} .

narrow ($\Delta\nu/c < 0.2$ cm^{-1}) laser excitation. An example of our results is shown in Fig. 1. When naphthalene in a frozen vitreous solvent is excited by a conventional $\Delta\nu/c \sim 1,000$ cm^{-1} source, the result is the normal emission spectrum (Fig. 1*a*). The vibrational bands, although well resolved, are broad, about 230 cm^{-1} for the 0.0 band. Excited in its 0.0 band at 4,765 Å by an argon ion laser, however, naphthalene exhibits the strikingly different spectrum (Fig. 1*b*). The emission line that occurs at about 4,830 Å is 3 cm^{-1} wide. The various sharp lines in Fig. 1*b* are allowed transitions from the lowest level of the excited state to the vibrational levels of the ground state. The broad band accompanying each line can be interpreted as the analogue of the phonon sidebands often observed in mixed-crystal spectra⁶. As a check, a vibrational analysis of the spectrum in Fig. 1*b* was carried out. The ground state vibrational frequencies thus obtained are in excellent agreement with those obtained for naphthalene by different methods⁷.

According to these results it seems natural to conclude that, at low temperatures under broadband excitation, naphthalene exhibits an inhomogeneously broadened

spectrum, one that is simply the superposition of many sharp spectra such as the spectrum in Fig. 1b. There is thus no intrinsic importance in either the width or the absolute location of the peaks in Fig. 1a, since they merely reflect the wide variety of environments provided for naphthacene by the particular vitreous host used. For this reason we have used brackets $\langle \rangle$ to signify an average value in labelling the principal peak in Fig. 1a.

If our interpretation of the origin of low temperature spectra is valid, excitation by very narrow band light in higher frequency absorption bands instead of the 0.0 band could lead to a much greater number of fluorescence lines (which may be resolvable under the appropriate conditions).

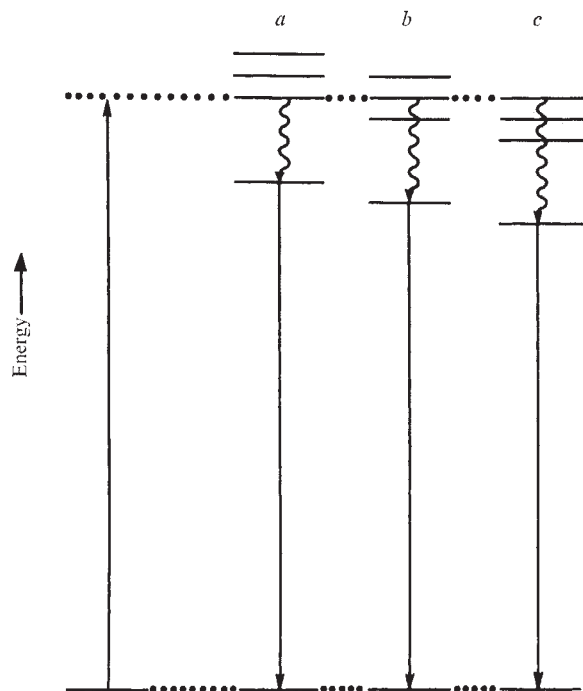


Fig. 2 Excitation outside the 0.0 band, showing accidental coincidence of higher vibronic lines due to different environments at site types labelled *a*, *b*, *c*. Wavy arrows indicate radiationless relaxation processes.

The reason for this is indicated in Fig. 2. Here we show hypothetical energy levels of three molecules identical in all respects except for their local environments, so that their electronic energy spacings differ. We have drawn the figure to show that narrow-band excitation could be resonant with all three molecules if the excitation were at the frequency shown. That is, excitation higher than the 0.0 band may lead to accidental coincidences and cause the fluorescence spectrum to be characteristic of several or many (in this example, three) environments at once.

Phenanthrene in methylcyclohexane typifies the multiple-line phenomenon. In Fig. 3 we show the result of very narrow-band excitation of phenanthrene at 3,250 Å, about 0.24 eV (220 Å) above the centre of the $\langle 0.0 \rangle$ absorption band. The spectrum does contain narrow lines, and they are more numerous than previously observed in mixed-crystal fluorescence spectra of phenanthrene⁸. The increased background is probably due to a superposition of the 'phonon' bands associated with all of the extra lines.

Studies of substituent effects on narrow-line spectra were also carried out for derivatives of two organic compounds, naphthacene and anthracene. In two parent aromatic hydrocarbons and four derivatives the spectra ranged from very broad and unresolved to very narrow lines. In particular, the broadening effect of phenyl groups, often

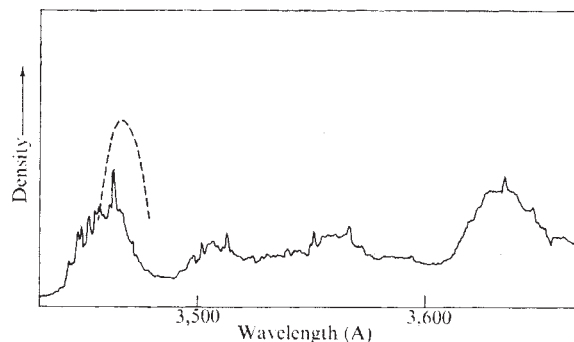


Fig. 3 The fluorescence spectrum of a 10^{-4} M solution of phenanthrene in methylcyclohexane glass at 4.2 K. Excitation is at 3,250 Å with a He-Cd ion laser. The excitation is 0.24 eV (220 Å) above the $\langle 0.0 \rangle$ band. The dotted curve is a portion of the $\langle 0.0 \rangle$ absorption band of phenanthrene in methylcyclohexane glass at 4.2 K.

observed at room temperature, remains substantial at 4 K even with laser excitation. Methyl groups were also found to broaden the spectra, whereas halogen substitution had no apparent effect.

We also examined a number of organic dyes. Although only broadband emission was obtained from ionic dyes, a few narrow lines were observed in a merocyanine.

We have also studied solvent effects in the production of narrow line spectra, including the effects of solvent polarity. Such compounds as naphthacene are only slightly sensitive to the polarity of the host solvent. The linewidth of the 313 cm^{-1} naphthacene fluorescence line is about 3 cm^{-1} in nonpolar methylcyclohexane, and about 5 cm^{-1} in the polar mixture of ethanol and methanol (1:1). Personov indicated a similar weak effect for perylene⁹. In azulene, which changes its dipole moment by 1.1 debye on excitation⁹, polar solvent broadening is, however, severe.

In summary, optical site selection in low temperature glasses provides a straightforward technique for observing vibronic structure normally obscured by inhomogeneous broadening. Typical inhomogeneous linewidths in these experiments are on the order of several hundred cm^{-1} . The homogeneously broadened 'zero-phonon' lines observed are about $3\text{--}10\text{ cm}^{-1}$ wide. Their measurement may prove to be a sensitive probe of the local solvent environment. To the extent that a low temperature glass represents a liquid solution at a fixed instant in time, studies of this nature may also provide insights into broadening mechanisms in liquid solutions.

We have begun a detailed examination of some of these effects using tunable laser excitation.

We thank K. H. Drexhage for helpful discussions. The work of two of us, J.H.E. and W.C.M., was partially supported by a grant from the United States Air Force Office of Scientific Research.

J. H. EBERLY
W. C. MCCOLGIN

Department of Physics and Astronomy,
University of Rochester,
Rochester, New York 14627

K. KAWAOKA
A. P. MARCHETTI

Research Laboratories,
Eastman Kodak Company,
Rochester, New York 14650

Received May 6; revised June 25, 1974.

¹ Meyer, B., *Low Temperature Spectroscopy*, chapters 1 and 2 (American Elsevier, New York, 1971).

² Craig, D. P., and Walmsley, S. H., *Excitons in Molecular Crystals*, chapter 6 (W. A. Benjamin, New York, 1968).

³ Shpol'skii E. V., *Soviet Phys. Uspekhi*, **6**, 411-427 (1963).

- ⁴ Szabo, A., *Phys. Rev. Lett.*, **25**, 924-926 (1970).
⁵ Personov, R. I., and Kharlamov, B. M., *Opt. Commun.*, **7**, 417-419 (1973).
⁶ Small, G. J., *J. chem. Phys.*, **52**, 656-673 (1970).
⁷ Kruse, N. J., and Small, G. J., *J. chem. Phys.*, **56**, 2985-2992 (1972).
⁸ Hochstrasser, R. M., and Small, G. J., *J. chem. Phys.*, **45**, 2270-2284 (1966).
⁹ Hochstrasser, R. M., and Noe, L. J., *J. chem. Phys.*, **50**, 1684-1688 (1969).

The origin of the far-infrared polarisation of liquid water

LIQUID water has a strong far-infrared polarisation. The origin of this has rarely been discussed in detail, although it causes the difference between the optical permittivity of about 1.8, and the limiting high frequency microwave (ϵ_∞) permittivity of about 4.2. The phenomenon has been attributed by Zafar *et al.*¹ to a high frequency relaxation of amplitude 2.4 in the permittivity, and a relaxation time of ~ 50 ns resulting from the orientation of water molecules that are free of hydrogen bonds. Unfortunately, the authors did not apply Sherlock Holmes² adage that when you have eliminated the impossible, whatever remains, however improbable, must be the truth. Not all possible explanations of this polarisation have been considered and eliminated, although the correct one has been mentioned. There can be little doubt that there is, at present, no evidence for a new orientational relaxation.

Another possible cause of the polarisation is, of course, resonance absorption, discussed briefly by Zafar *et al.*¹ in terms of a single damped resonance absorption centred at 170 cm^{-1} . It may not be possible to describe, however, a resonance absorption band using a single damped resonator; there may be a continuous distribution of resonators, and any arbitrary distribution of intensity may occur, even one that mimics a relaxation absorption. Relaxation and broad resonance absorptions cannot be distinguished by direct measurement, and so, to decide which is occurring in liquid water, the spectrum of water can be compared with that of ice, which has no relaxation absorption in this frequency region.

Ice I has an infrared polarisation responsible for the difference between the optical and microwave dielectric constants: 1.8 and 3.1, respectively³. The difference, Δn , between the corresponding refractive indices is proportional to the negative second moment of the absorption spectrum:

$$\Delta n = \frac{1}{2}\pi^{-2} \int_{\text{band}} K(v) v^{-2} dv$$

where $K(v)$ is the absorptivity at wave number v . In ice, the main contributory bands are the H_2O internal motions in the range $3,600\text{--}1,000\text{ cm}^{-1}$, the H_2O rotational vibrations centred in the infrared at 850 cm^{-1} , and the H_2O translational vibrations centred at 229 cm^{-1} . They contribute, respectively, 0.0506, 0.0728 and 0.347 to the infrared component of the low frequency refractive index³.

Internal motions contribute little in ice; they contribute even less in water, because the O-H stretching frequencies are higher. They might contribute, say 0.05 to the refractive index. The rotational vibrations in ice extend from 525 to $1,040\text{ cm}^{-1}$, the infrared intensity being centred at $\sim 850\text{ cm}^{-1}$. The effective dipole moment is $1.45 D$ (ref. 4), compared with the value for the vapour of $1.84 D$. In the liquid there is a broad band because of these vibrations. Its peak is shifted from 850 cm^{-1} , the frequency in ice, to about 650 cm^{-1} , and it is greatly broadened, the lower limit decreasing from 525 cm^{-1} to 300 cm^{-1} or less. The negative second moment is, therefore, about twice as great for the liquid as for ice, and so its contribution to the low frequency refractive index is about 0.15. A more accurate value may be obtainable from the measured spectrum, but it would be difficult to separate the overlapping rotational and translational bands with any reasonable certainty.

Absorption in ice, which results from the translational vibrations, extends from 318 cm^{-1} to zero frequency⁵ and its maximum is at about 229 cm^{-1} . The integrated intensity⁶ shows that the effective charge for the stretching of the hydrogen bond is about $0.4e$, where e is the electronic charge.

The band is broadened and the peak shifted to about 220 cm^{-1} in vitreous ice⁷, and undoubtedly persists in the liquid, because the diffusion coefficient, the viscosity, and the relaxation of molecular and nuclear orientational polarisation⁸ indicate that a water molecule requires about 10 ps to change its position or orientation at 25°C , whereas a frequency as low as even 30 cm^{-1} corresponds to about 1 vibration ps^{-1} . It is no doubt broadened greatly by the increased disorder of the liquid as compared with ice and by the short lifetime of a state. In addition, the stretching force constant of the hydrogen bonds undoubtedly takes on a range of values because of the range of hydrogen bonds. All of these processes broaden the band and increase its negative second moment. Because of the increased length of the hydrogen bond, the force constants are undoubtedly less in water than in ice, and that will cause the whole band to shift to low frequency, and will further increase the second moment. The band has not been analysed in detail, but the peak intensity is at about 170 cm^{-1} (see ref. 9 and refs quoted there). If the whole ice band were shifted by the ratio 170:229, the negative second moment would be doubled, and the contributions to the low frequency refractive index would be doubled to about 0.7. The discussed broadening would further increase the contribution. The increased length of the hydrogen bond might then decrease the effective charge, thus reducing the contribution to the refractive index.

It seems likely that the internal rotational and translational resonance absorption contribute somewhat less than about 0.9 to the low frequency refractive index, which corresponds to a value for ϵ_∞ of somewhat less than about 5, compared with the experimental value of 4.2.

It seems, therefore, that there is little or no room for a relaxation absorption, and that if it does occur, it is at least an order of magnitude less than that assumed by Zafar *et al.*¹, and is strongly overlapped by resonance absorption.

This conclusion is confirmed by the $\text{H}_2\text{O}\text{--}\text{D}_2\text{O}$ isotope effect in the spectrum. If there were a relaxation absorption because of the rotation of molecules, the relaxation times for D_2O and H_2O would have an approximate ratio of 1.4. The relaxation times deduced by Zafar *et al.* are in the ratio 0.85. Absorption because of damped translational vibrations, for which corresponding features would be at frequencies and absorptivities in the ratio 1.05, is more consistent with the measurements summarised by Zafar *et al.*¹ when allowance is made for the effects of the well established orientational relaxation. Both the infrared and Raman spectra of H_2O and D_2O have bands centred at about 170 cm^{-1} , and there is a Raman band at about 60 cm^{-1} (ref. 10). These no doubt result from damped translational vibrations; not from rotational motions.

E. WHALLEY

Division of Chemistry,
National Research Council of Canada,
Ottawa, Canada K1A 0R9

Received February 18; revised July 11, 1974.

- ¹ Zafar, M. S., Hasted, J. B., and Chamberlain, J., *Nature phys. Sci.*, **243**, 106 (1973).
² Doyle, A. C., *The Sign of Four*.
³ Bertie, J. E., Labbé, H. J., and Whalley, E., *J. chem. Phys.*, **50**, 4501 (1969).
⁴ Ikawa, S.-I., and Maeda, S., *Spectrochim. Acta*, **24A**, 655 (1968).
⁵ Whalley, E., and Labbé, H. J., *J. chem. Phys.*, **51**, 3120 (1969).
⁶ Klug, D. D., and Whalley, E., in *Physics and Chemistry of Ice* (edit. by Whalley, E., Jones, S. J., and Gold, L. W.), 93 (Royal Society of Canada, Ottawa, 1973).
⁷ Bertie, J. E., and Whalley, E., *J. chem. Phys.*, **46**, 1271 (1967).

- ⁸ Eisenberg, D., and Kauzmann, W., *The structure and properties of water*, 205. (Clarendon Press, Oxford, 1969).
⁹ Robertson, C. W., Curnutte, B., and Williams, D., *Molec. Phys.*, **26**, 183 (1973).
¹⁰ Walrafen, G. E., *Water: A comprehensive treatise*, vol. 1 (edit. by Franks, F.), chapter 5 (Plenum Press, New York, 1972).

DRS HASTED, ZAFAR AND CHAMBERLAIN REPLY: Although he has cast us¹ in the role of Inspector Lestrade², Dr Whalley³ performs a useful service by questioning our neglect of resonance absorptions in the discussion of the enhanced permittivity increment, $\epsilon_\infty - n^2$, of water. Complete analysis of the origins of this increment must await the collection and analysis of refractive index data of improved accuracy over a much wider frequency range than has so far been investigated; but in the meantime we point out that the application of the Kramers-Kronig relationship to absorption data alone, can be confusing. The integrated negative second moment of the power absorption coefficient contributes proportionally not to ν^{-2} but to ν^{-1} , so that a shift of the absorption band by a factor of 229:170 alters its contribution to n^2 , not by 235% but by 83%. Further, the maximum of the liquid water band taken to be at 170 cm^{-1} is now placed as high as 200 cm^{-1} (ref. 4).

In liquid water, both the 683 cm^{-1} and the 200 cm^{-1} band contribute to the $\Delta\epsilon'$ increment just as they do in ice. They arise from intermolecular librational resonance processes. Normal coordinate analysis^{4,5} has been used to assign the 683 cm^{-1} band to libration of the symmetry species B_2 of the $(\text{H}_2\text{O})_5$ pentamer, and the 200 cm^{-1} band to the translational modes of species A_1 , B_1 and B_2 . Both processes are resonant rather than relaxational, and exhibit an S-shaped 'anomalous dispersion'⁶ in ϵ' . Libration processes also contribute an increment $\epsilon' \propto \mu^2/I\nu^2$, according to Poley⁷. Note that it is to ϵ' and not to n that there is a contribution proportional to ν^{-2} . This type of absorption has been called 'Poley absorption', and is found in many unassociated organic liquids in the submillimetre region (for example ref. 8). Apparently, no band corresponding to free molecules is found in water or in alcohols⁹, with the exception of *t*-butyl alcohol, in which there seems to be less association. (J. C., G. J. Davies, J.B.H. and M.S.Z., unpublished data.)

We believe that there are only a small proportion of free molecules in liquid water, and that their reorientation is a relaxation rather than a resonance process. The extraordinarily large bandwidth does not seem to be strongly temperature dependent, as would be the case for a resonance process; nor is the centre of the band dependent upon I , the molecular moment of inertia.

There is, as yet, no evidence to rule out the presence of an underlying continuum such as may be contributed by a relaxation process, although its extent and magnitude may not be as large as would at first seem. Certainly, the absence of a second relaxation in ice should not lead us to anticipate its absence in liquid water. Resonance processes in the submillimetre band are often accompanied by an actual minimum in the refractive index¹⁰, or in the permittivity function, and Poley absorption may be of this type. This phenomenon may also contribute in liquid water in the 1–100 cm^{-1} range. Our double Debye analysis neglects such a contribution and leads to a discrepancy in ϵ_∞ (ref. 1, Fig. 6) which could perhaps be resolved if it were taken into account.

No relaxation isotope effect, $\tau(\text{D}_2\text{O})/\tau(\text{H}_2\text{O}) = 1.4$, is to be expected, because the molecular moment of inertia, although involved in resonance rotation, is not involved directly in relaxation theory. For example, it does not appear in the water principal relaxation as interpreted on the Debye-Stokes theory¹¹. The probable error on both proposed second relaxation times is very large, and the ratio could lie anywhere between 0.5 and 1.5.

We do not wish to imply that the resonance processes can be neglected in comparison with the relaxation of free molecules, as could have been concluded from our ambitious attempt to construct a Cole-Cole diagram between ϵ_∞ and n_D^2 . Rather,

the increment must be a summation:

$$\epsilon_\infty - n_D^2 = \Delta\epsilon' (\text{libration}) + \Delta\epsilon' (\text{translation}) + \Delta\epsilon' (\text{relaxation})$$

Birkbeck College and
National Physical Laboratory.

- ¹ Zafar, M. S., Hasted, J. B., and Chamberlain, J., *Nature phys. Sci.*, **243**, 106 (1973).
² Doyle, A. C., *The Adventures of Sherlock Holmes*.
³ Whalley, E., *Nature*, **251**, 217 (1974).
⁴ Curnutte, B., and Williams, D., *Proc. int. Symp. Structures of Water and Aqueous Solutions*, Marburg, 1973 (edit. by Luck, W.), (Physik Verlag, Baden, 1973).
⁵ Bryan, J. B., and Curnutte, B., *J. molec. Spectrosc.*, **41**, 512 (1972); Bryan, J. B., thesis, Kansas State Univ. (1969).
⁶ Querry, M. R., Curnutte, B., and Williams, D., *J. opt. Soc. Am.*, **59**, 1299 (1969).
⁷ Poley, J. Ph., *J. chem Phys.*, **23**, 405 (1955).
⁸ Davies, G. J., and Chamberlain, J., *J. chem. Soc., Faraday Trans. II*, **69**, 1739 (1973).
⁹ Davies, G. J., thesis, Univ. Wales (1971).
¹⁰ Chamberlain, J., *Chem. Phys. Lett.*, **2**, 464 (1968).
¹¹ Collie, C. H., Hasted, J. B., and Ritson, D. M., *Proc. phys. Soc.*, **60**, 145 (1948).

Black polar bears

WHITE coloration of many animals in cold environments decreases the possibility of visual detection because of the lack of contrast between the animal and its white background. White hair thus provides concealing coloration for prey species such as arctic hare (*Lepus arcticus*), and for predators such as the polar bear (*Ursus maritimus*) and arctic fox (*Alopex lagopus*).

Such animals often live in areas where aerial detection is feasible because of the lack of overhead cover, yet present remote sensing techniques do not usually distinguish these white animals against a white background. Thermal infrared imagery will detect some white animals, including the polar bear¹ and white-coated pups of the grey seal (*Halichoerus grypus*) (N.A.Ø., and D. M. L., unpublished) but only in certain weather conditions.

When cold and windy, sufficient temperature differences may not exist between a well insulated animal and its environment to enable detection by a thermal scanner.

Our preliminary attempts to improve detection of white animals on snow suggested that photography in the near ultraviolet band (300–400 nm) of the electromagnetic spectrum markedly increased contrast between some white animals and their backgrounds².

We recently obtained oblique photographs of polar bears on the tundra south of Churchill, Manitoba, Canada. The low visual contrast of a polar bear on snow is shown by black and white photography in the visible region of the electromagnetic spectrum (Fig. 1a). Ultraviolet photography, however, resulted in a black image of the polar bear against white snow (Fig. 1b).

This difference in the ultraviolet absorbance and reflectance characteristics of the polar bear pelt and snow has direct applications for remote sensing of polar bears in the field. Although the distance between polar bears on the tundra makes photographic surveys impractical over wide areas of the arctic, ultraviolet photography may be useful for aerial detection and censusing bears in specific areas, where they congregate at certain times of the year, or along known migration routes. The ultraviolet band may also be used in long range detection and observation of bears on ice. At present, such observations, often made by telescope, are also hindered by the lack of contrast between the bear and its environment. Contrast may be enhanced by coupling an ultraviolet transmitting lens covered by a visibly opaque, ultraviolet transmitting filter, with an image intensifier whose spectral sensitivity extends into the near ultraviolet region of the electromagnetic spectrum.



Fig. 1 Photography of a polar bear (*Ursus maritimus*) and three cubs on tundra. *a*, Normal black and white photograph in the visible portion of the electromagnetic spectrum (Kodak Double-X Aerographic 2405 film, Hasselblad 150 mm Sonnar lens, $f/16$, $1/500$ s). *b*, Ultraviolet photograph (Kodak Aerographic Double-X 2405 film, Hasselblad 105 mm UV-Sonnar lens, Kodak 18A filter, $f/5.6$, $1/500$ s).

This observation of 'black' polar bears seems to have further implications. At present, the biological significance of white coat coloration in animals is not clear. Whiteness in arctic climates obviously provides camouflage for many birds and mammals. Theoretical analysis and quantitative measurements suggest that some white pelts transfer solar energy more efficiently to the skin than dark pelts^{3,4}. Thus white coloration may also play an important role in thermoregulation for a number of species including the white-coated pups of the harp seal (*Pagophilus groenlandicus*)⁵. The older view that dark pelts absorb solar radiation while white pelts reflect it and thus reduce radiation absorption⁶ is probably incorrect. Regardless, the fact that some white pelts absorb ultraviolet radiation while others, such as the arctic hare reflect it², suggests that any discussion of "life's color code"⁶ is incomplete without further consideration of the near ultraviolet component in solar radiation.

We thank Drs I. Stirling, C. Jonkel, and K. Ronald for their support.

D. M. LAVIGNE
N. A. ØRITSLAND

Department of Zoology,
College of Biological Science,
University of Guelph,
Guelph, Ontario, Canada

Received June 3, 1974.

¹ Brooks, J. W., in *Bears—their biology and management* (edit. by Herrero, S.), 138–141 (IUCN, Morges, Switzerland, 1972).

² Lavigne, D. M., and Øritsland, N. A., *Can. J. Zool.* (in the press).

³ Kovarik, M., *Nature*, **201**, 1085 (1964).

⁴ Øritsland, N. A., *Comp. Biochem. Physiol.*, **40A**, 359 (1971).

⁵ Øritsland, N. A., and Ronald, K., *Comp. Biochem. Physiol.*, **44A**, 519 (1973).

⁶ Hamilton, W. J. III, *Life's color code*, 49–67 (McGraw-Hill, New York, 1973).

Metabolic hydroxylation of a chlorobiphenyl containing only isolated unsubstituted positions—2,2',4,4',5,5'-hexachlorobiphenyl

RECENT investigations on the metabolism of chlorobiphenyls of known structure have shown that compounds containing four chlorine atoms or less are hydroxylated

by rats, rabbits and pigeons^{1–3}. All the compounds investigated have two unsubstituted carbon atoms in ortho positions to each other. It has been suggested that this substitution pattern is required for the metabolic breakdown of chlorobiphenyls⁴. A thorough investigation of the chlorobiphenyl residues found in man and in higher animals partly confirms this suggestion⁵.

Hutzinger *et al.*² have investigated the fate of a chlorobiphenyl containing only isolated, unsubstituted carbon atoms—2,2',4,4',5,5'-hexachlorobiphenyl—in rats, pigeons and trout. No hydroxy-containing metabolites were detected in the excreta by the methods used. This chlorobiphenyl is one of the major components in technical PCB with chlorine contents above 50%. Furthermore, it is found in relatively high amounts in all species investigated including man⁷.

It has been shown, however, that rabbits given 1,2,3-, 1,2,4- or 1,3,5-trichlorobenzene always excreted chlorinated monophenols. The yields over 5 d were 78, 42 and 5%, respectively⁸.

The fact that even 1,3,5-trichlorobenzene (containing only isolated unsubstituted carbon atoms) is hydroxylated, led us to reinvestigate the behaviour of 2,2',4,4',5,5'-hexachlorobiphenyl in some animal species.

Rats were given the chlorobiphenyl⁹ in peanut oil (20 mg ml⁻¹) and an amount corresponding to about 15 mg was consumed during the night by each animal. Faeces were collected daily, dried and extracted with ether (Soxhlet extraction). The extracts were divided into two parts, one of which was treated with diazomethane. After evaporation of solvent the residue was dissolved in hexane and shaken with sulphuric acid before analysis by gas chromatography using an electron capture detector⁷. The acid treatment removes most unwanted extractives but does not affect chlorobiphenyls and methoxy-chlorobiphenyls.

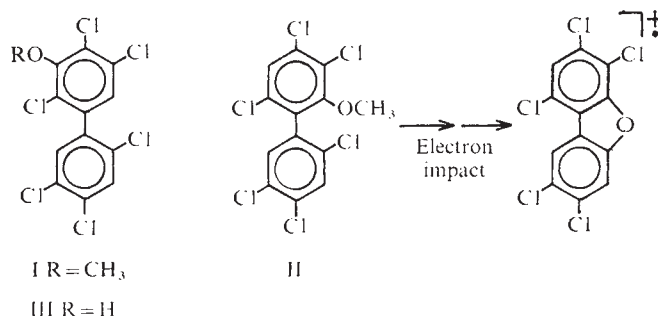


Fig. 1 Alternative structures I and II of the methylated metabolite of 2,2',4,4',5,5'-hexachlorobiphenyl in rats and mice. The suggested cyclisation reaction (see text) of II upon electron impact in the mass spectrometer is indicated.

In addition to unchanged hexachlorobiphenyl a new compound was found in the methylated extract after 2 d. When analysed by combined gas chromatography-mass spectrometry (quadropole instrument) this substance showed a molecular ion at 388 mass units (m.u.) (6 Cl) corresponding to a methoxyhexachlorobiphenyl. The structure of this compound (Fig. 1), obviously derived from a phenolic metabolite of 2,2',4,4',5,5'-hexachlorobiphenyl, must be I or II provided no migrations of chlorine atoms have taken place.

Mass spectral studies performed in this laboratory using about 35 synthetic methoxychlorobiphenyls (S. J. and G. S., unpublished) have shown that compounds containing a methoxy group in the two position, II, for example, readily lose CH₃Cl (50 m.u.) upon electron impact. This results in very abundant peaks in the range 83–234% of the molecular ion at M⁺-50 m.u. which can be explained by the formation of cyclic structures (Fig. 1).

Similar fragmentation patterns have previously been

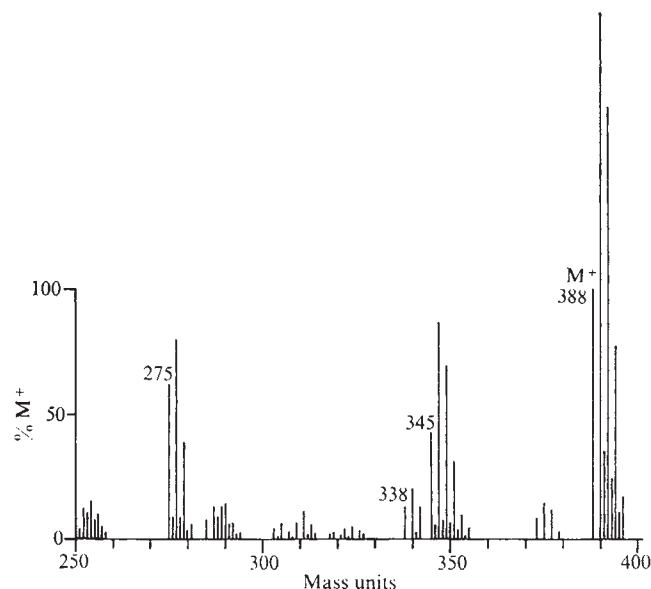


Fig. 2 Part of the mass spectrum of the methylated metabolite.

observed in the mass spectra of 2-methoxychlorodiphenyl ethers^{10,11}.

The loss of CH_3Cl in the mass spectra of all 3- or 4-methoxychlorobiphenyls investigated so far have on the other hand, never exceeded 30% of the molecular ion. The mass spectrum of the methyl ether of the present metabolite (Fig. 2) shows a peak at $M^+ - 50$ m.u. which is only 13% of the molecular ion. This fragmentation strongly favours structure III of the original phenol. Final proof for the structure, however, must await comparison with synthetic material of known structure.

The elimination rate of unchanged hexachlorobiphenyl and the metabolite (assuming equal detector response on GLC) is shown in Fig. 3. The total amount of metabolite excreted during 7 d was calculated to be about 1.3% of the dose. The corresponding figure for unchanged biphenyl was about 7%. No trace of III or conjugates thereof were found in the urine.

Our experiments do not discriminate between microsomal or microbial hydroxylation of the chlorobiphenyl as no bile cannulation experiments have been performed. But the presence of the metabolite in the faeces—and not in the urine—is in accordance with the results of other investigations concerning the metabolism of PCB in rats^{3,4}.

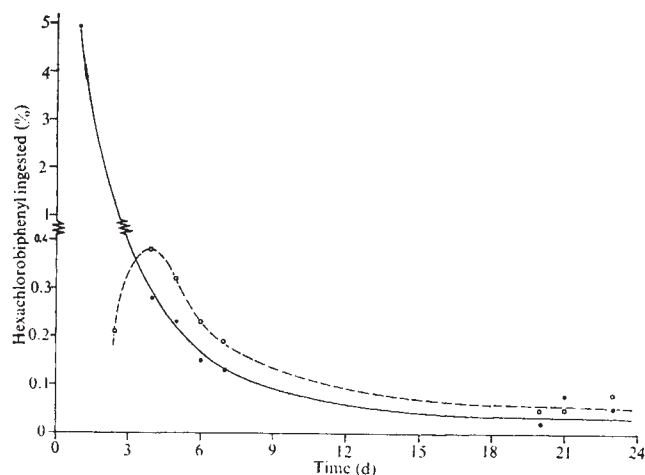


Fig. 3 Excretion rate (faeces) of 2,2',4,4',5,5'-hexachlorobiphenyl (—) and its metabolite (---) from a rat as % hexachlorobiphenyl ingested.

Control experiments showed that bacterial action during the drying of the faeces was not responsible for the hydroxylation.

Mice given the hexachlorobiphenyl also excreted III (GLC) in the faeces. When the substance was given to quails (about 11 mg per animal) no metabolite could be detected in the excreta. It was, however, found that large amounts of unchanged chlorobiphenyl were excreted in both faeces and eggs—about 9 and 33%, respectively, during 10 d.

The results presented here show that a chlorobiphenyl containing only isolated unsubstituted positions can be metabolised by mammals albeit at a lower rate than chlorobiphenyls containing vicinal hydrogen atoms. It seems, therefore, that most of the high chlorinated compounds in PCB can be excreted not only unchanged but also as metabolites. The latter route implies that the chlorobiphenyls are not further circulated in the environment but that new compounds are released the persistence and toxicity of which are not sufficiently known.

This work was supported by the National Swedish Environment Protection Board. We thank Drs J. Kihlström and J. Öhrborg supplying the excreta from the mice and Dr C. A. Wachtmeister for discussions. Mass spectra were run by Dr B. Jansson.

S. JENSEN

National Swedish Environment Protection Board,
Special Analytical Laboratory

G. SUNDRÖM

Environmental Toxicology Unit,
Section of Organic Chemistry,
Wallenberg Laboratory,
University of Stockholm,
S-104 05 Stockholm 50

Received April 8; revised June 7, 1974.

- ¹ Block, W. D., and Cornish, H. H., *J. biol. Chem.*, **234**, 3301–3302 (1959).
- ² Hutzinger, O., Nash, D. M., Safe, S., DeFreitas, A. S. W., Norstrom, R. J., Wildish, D. J., and Zitko, V., *Science*, **178**, 312–314 (1972).
- ³ Yoshimura, H., and Yamamoto, H., *Chem. pharm. Bull.*, **21**, 1168–1169 (1973).
- ⁴ Yoshimura, H., Yamamoto, H., and Saeki S., *Chem. pharm. Bull.*, **21**, 2231–2236 (1973).
- ⁵ Gardner, A. M., Chen, J. T., Roach, J. A. G., and Ragelis, E. P., *Biochem. biophys. Res. Commun.*, **55**, 1377–1384 (1973).
- ⁶ Schulte, E., and Acker, L., *Naturwissenschaften*, **61**, 79 (1974).
- ⁷ Jensen, S., and Sundström G., *Ambio*, **3**, 70–76 (1974).
- ⁸ Jondorf, W. R., Parke, D. V., and Williams, R. T., *Biochem. J.*, **61**, 512–521 (1955).
- ⁹ Moron, M., Sundström, G., and Wachtmeister, C. A., *Acta chem. scand.*, **27**, 3121–3122 (1973).
- ¹⁰ Jensen, S., and Renberg, L., *Ambio*, **1**, 62–65 (1972).
- ¹¹ Rappe, C., and Nilsson, C.-A., *J. Chromat.*, **67**, 247–253 (1972).

Sexual maturation of Japanese eel and production of eel larvae in the aquarium

THE life history of the eel is complex and despite many investigations little is known about its embryonic development. Some investigators such as Grassi and Calandriccio (1896: cited in ref. 1) and Fish¹, described the supposed early development of eels, using developing eggs collected by a trawl for young fish in suspected spawning grounds of eels. The time of fertilisation of these eggs, however, was unknown, and the species involved was uncertain. Artificial maturation of eels by hormonal treatment is another approach which has been attempted by many workers^{2–6}. They all, however, failed to obtain mature eggs and sperm and thus to clarify the embryonic development of eels. Here we describe the first success with this approach.

Table 1 Early development of Japanese eel at 23° C

Time after fertilisation	Stage	Remarks
20–50 min	Single cell	Pelagic, with appearance of perivitelline space; egg diameter was 1.3 mm.
80 min–3½ h	Cleavage	Typical discoidal cleavage.
3½–5½ h	Morula	Three to five layers of blastomeres.
5½–9 h	Blastula	Blastocoel discernible from outside.
9½–12 h	Gastrula	Blastoderm covering ¼ of yolk.
13–18 h	Embryonic body formation	Blastoderm covering ½ to ¾ of yolk.
19–24 h	Blastopore closure	Differentiation of head and 3 to 12 somites.
25–27 h	Eye vesicle and ear vesicle formation	Tail bud formation.
28–33 h	Heart formation	About 32 somites.
35–37 h	Start of heart pulsation	Embryo covering entire yolk.
38–45 h	Hatching	Membranous fin well developed; no pectoral fins visible.

Silver eels of *Anguilla japonica* were caught in the Mabuchi and Hiranuma rivers in Aomori prefecture (Japan) during September 1973. They were transferred to Hakodate and raised in circulating purified seawater tanks with a capacity of 5.5 gallons. Male eels were injected with Synahorin, a mixture of pituitary gonadotrophin and chorionic gonadotrophin (Teikoku Zoki), and the females were injected with saline suspension of chum salmon pituitary as previously reported^{7,8}. More than

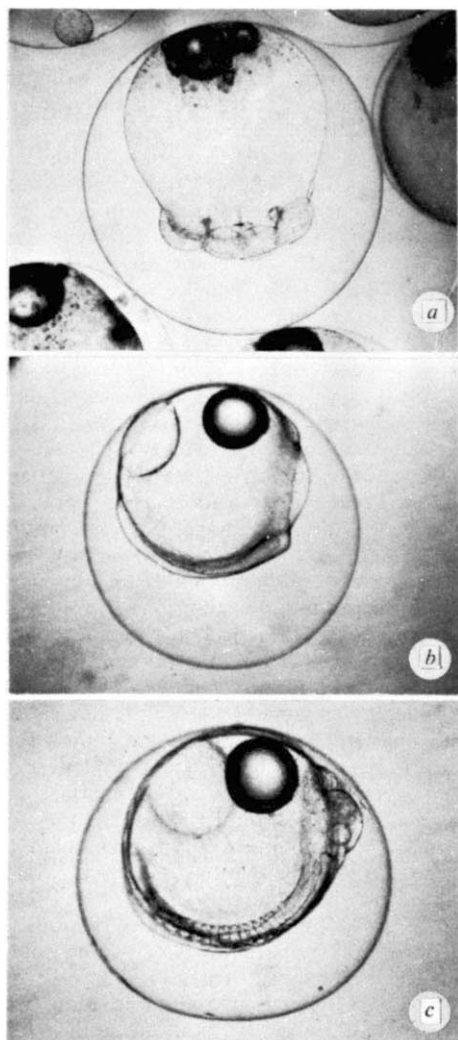


Fig. 1 Living eggs at, *a*, eight-cell stage, $\times 30$; *b*, closing-blastopore stage, $\times 30$; *c*, stage of eye vesicle formation, $\times 30$.

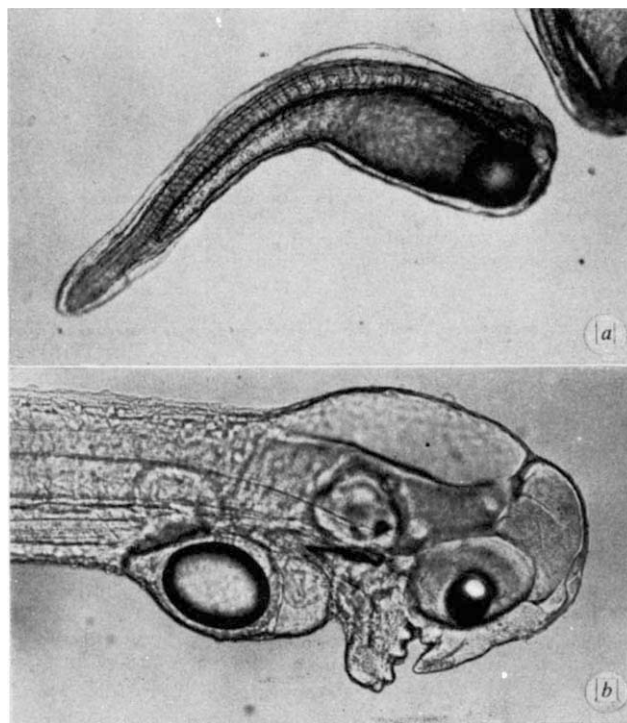


Fig. 2 Living larvae: *a*, 30 min after hatching, $\times 28$; *b*, head of larvae, fifth day after hatching, $\times 70$.

20 males and five females became fully sexually mature following these treatments. Eggs and milt were stripped from the fish into glass dishes; fertilisation occurred, and the development of the eggs in normal seawater at 23° C was observed. The spherical ripe eggs (Fig. 1) were white and transparent; they measured about 1.0 mm in diameter. The development of the eel egg is summarised in Table 1.

Hatched larvae (Fig. 2) survived for about 5 d. They measured about 4.8 mm, 5.3 mm, 5.9 mm and 6.2 mm in body length on the second, third, fourth and fifth days after hatching, respectively. By the sixth day, when the yolk sac of larvae was almost absorbed, most larvae showed curvature of the tail and then died. Some surviving larvae measured about 5.8 mm in average length. By the third day after hatching, the mouth and anus of the larvae were open, and the pectoral fins also appeared. The brain differentiated into fore, mid and afterbrain, showing a large fourth ventricle. From the fifth to sixth day, the jaw of the larvae developed conspicuously and tooth anlagen characteristic of muraenoid larvae appeared. Fifty-three pre-anal and about 48 postanal myomeres were counted in the sixth day larvae.

These observations allow us to correct and complete earlier claims.

We thank Drs H. Takahashi and K. Takano, and the students in the Laboratory of Freshwater Fish Culture, Faculty of Fisheries, Hokkaido University, for their help. We thank Professor H. A. Bern, Department of Zoology, University of California, Berkeley, for reading the manuscript. This work was supported by a grant from Japanese Ministry of Education.

K. YAMAMOTO
K. YAMAUCHI

Faculty of Fisheries,
Hokkaido University,
Hakodate, Japan

Received April 18; revised June 25, 1974.

¹ Fish, M. P., *Zoologica*, **8**, 287–424 (1927).

² Bruun, A. F., Hemmingsen, A. M., and Møller-Christensen, E., *Acta Endocrinol.*, **2**, 212–226 (1949).

- ³ Møller-Christensen, E., Bruun, A. F., and Hemmingsen, A. M., *Acta Endocrinol.*, **28**, 103-111 (1958).
⁴ Boetius, J., Boetius, I., Hemmingsen, A. M., Bruun, A. F., and Møller-Christensen, E., *Medd. Danm. Fisk.-og Havunders.*, **3**, 183-198 (1962).
⁵ Fontaine, M., Bertrand, E., Lopez, E., and Callamand, O., *C.r. Seanc. hebdom. Acad. Sci. Paris*, **259**, 2907-2910 (1964).
⁶ Ohkami, H., and Iizuka, M., *Rep. Shizuoka Fish. exp. Stat.*, 140-142 (1965).
⁷ Yamamoto, K., Hiroi, O., Hirano, T., and Morioka, T., *Bull. Jap. Soc. scient. Fish.*, **38**, 1083-1090 (1972).
⁸ Yamamoto, K., Morioka, T., Hiroi, O., and Omori, M., *Bull. Jap. Soc. scient. Fish.*, **40**, 1-7 (1974).

A long continuous pollen sequence from north-eastern Australia

FEW pollen diagrams from Australian Quaternary deposits have been published and none of these extends beyond 10,000 BP. In north-eastern Australia, Lake Euramoo¹ provides a pollen record from 9,700 BP to 1,500 BP and Quincan Crater² gives evidence of vegetation changes from 7,250 BP to the present day. Here I outline results from a third site on the Atherton Tableland, Lynch's Crater, which provides a much longer vegetation record.

Lynch's Crater is of volcanic origin, about 600 m in diameter and filled with organic lake and swamp deposits to 45 m below the crater rim. The crater wall has been breached and further swamp development is thereby prevented. In comparison with previously described sites (Fig. 1), Lynch's Crater receives a much higher mean annual rainfall (approximately 2,500 mm), though the crater is at a similar altitude (760 m) and is also located within the basalt-covered Tableland. Under undisturbed

conditions, Lynch's Crater was surrounded by Complex Mesophyll Vine forest (terminology from ref. 3), whereas within the area as a whole, rain forest occurs on all well-drained soils with a mean annual rainfall greater than 1,400-1,500 mm. Under lower rainfalls and on soils with impeded drainage, sclerophyll vegetation, normally dominated by species of *Eucalyptus*, or *Melaleuca*, replaces rain forest.

A core taken near the centre of the swamp provided 20 m of organic sediments for pollen analysis and radiocarbon dating. Selected results of pollen analyses at intervals along the core are shown in diagrammatic form on Fig. 2. Three distinct periods can be recognised. Zones F, E and D contain high percentages of the rain forest gymnosperms, *Podocarpus* and *Araucaria* and, except for zone E, low values of rain forest angiosperms; zone B is dominated by the sclerophyll taxa *Casuarina* and *Eucalyptus* comp.; zone A is mainly composed of rain forest angiosperm pollen. Zone C is transitional between earlier rain forest and later sclerophyll assemblages. The total number of dry land taxa recorded in each sample is highest in zones E and A and lowest in zone B.

Much of the pollen sequence is beyond radiocarbon dating range. It is difficult to extrapolate the age of the sediment to the base of the core because of variations in sediment type and probably in sedimentation rates, but it may lie between 60,000 and 70,000 years BP. It is suspected that the youngest date ($6,850 \pm 90$ b.p. ANU-958), which corresponds with the zone A/B boundary, may not be accurate, since it could be contaminated by roots younger than the pollen analysed matrix.

Interpretation of the pollen diagram relies heavily on comparisons of the fossil spectra with spectra from surface samples collected within a variety of rain forest and sclerophyll vegetation types in north-east and south-east Queensland⁴.

Pollen spectra of zone F correspond with moist araucarian forests (Low Microphyll Vine forests with *Araucaria*) which are extensive in southern Queensland but hardly present in the north-east of the state today. *Araucaria* is a common emergent above subtropical rain forest canopies on basaltic soils where the mean annual rainfall lies between 900 and 1,400 mm (L. J. Webb, communicated at Symposium on Ecological Research in Humid Tropics Vegetation, Kuching, 1965). It is therefore likely that rainfall was less than half the 2,500 mm received today during the period represented by zone F, though mean annual temperatures may have been little different from present day values. In zone E, higher percentages of Urticaceae/Moraceae and *Elaeocarpus* pollen at the expense of pollen from rain forest gymnosperm and sclerophyll taxa, as well as an increase in the total number of dry land taxa represented, indicate an increase in complexity of the surrounding vegetation. Complex Notophyll Vine forest with *Araucaria* probably replaced Low Microphyll Vine forest with *Araucaria* under increased precipitation (approximately 1,400 mm yr⁻¹). Zone D is very similar floristically to zone F and this suggests a return to Low Microphyll Vine forest under reduced rainfall.

The transition from Low Microphyll Vine forest to Sclerophyll vegetation occurred between about 38,000 and 27,000 years BP. The reason for this change could be a further decrease in precipitation but man, with his use of fire, may also have had an important effect, for it is now known that he has been in Australia for at least the past 32,000 yr (ref. 5). During the period of maximum sclerophyll expansion, rain forest patches which are not extensive today along the east coastal areas of Australia must have been very much restricted in area, and many rain forest types probably survived in stream gullies and other favourable retreats.

At some time before 7,000 BP, rain forest again invaded

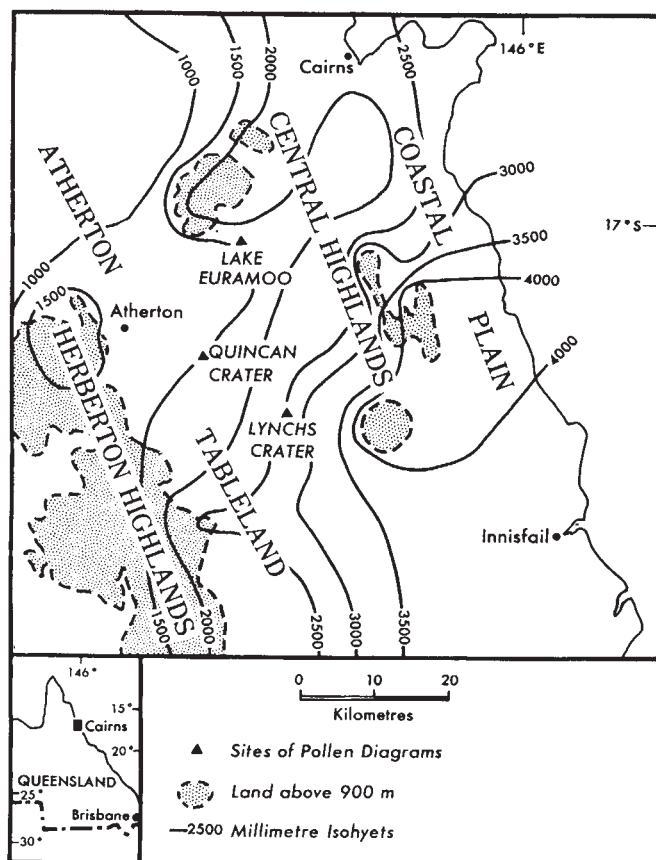


Fig. 1 Location of Lynch's Crater.

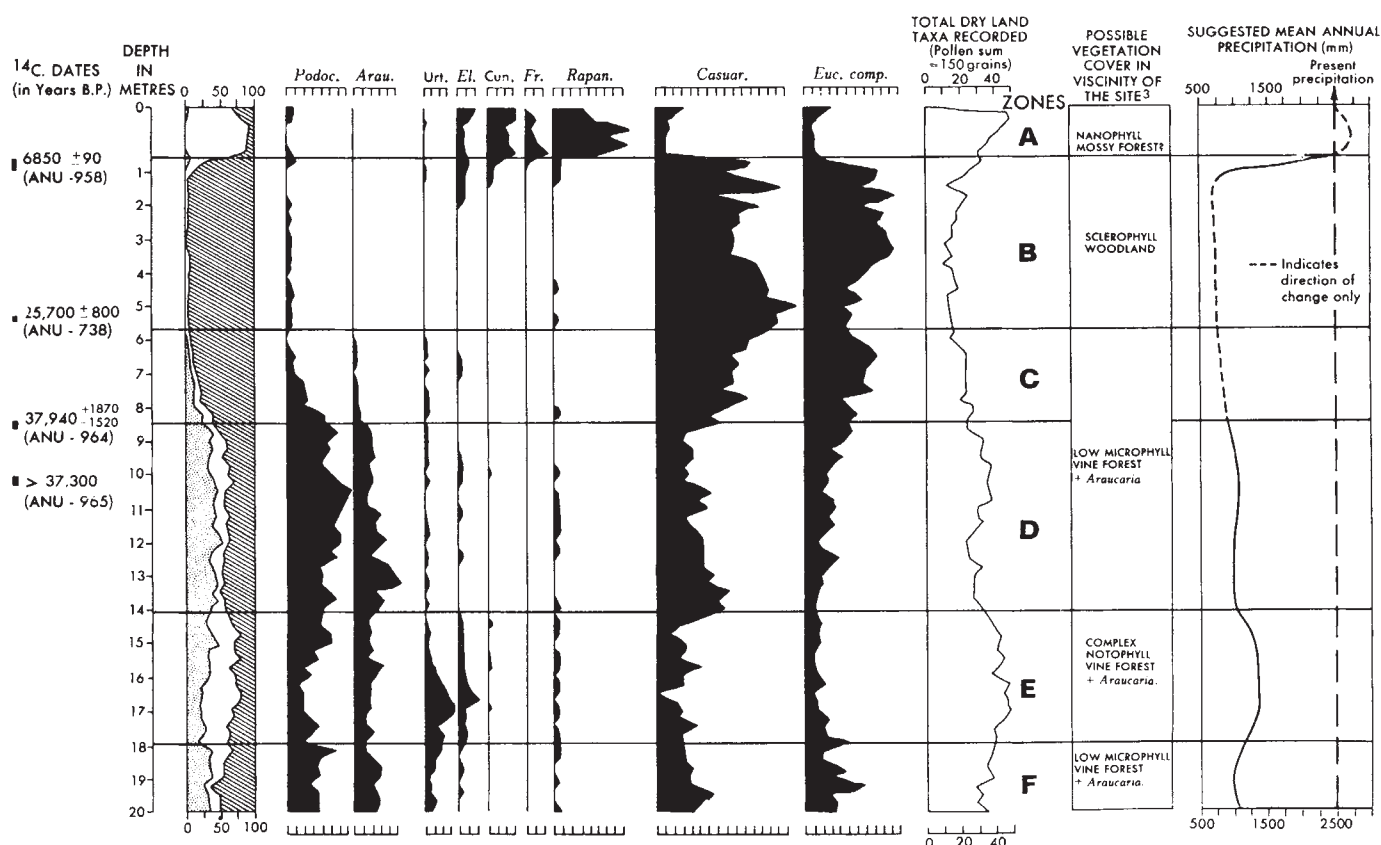


Fig. 2 Pollen diagram from Lynch's Crater. The column on the left shows the percentage of rain forest gymnosperms (stippled); rain forest angiosperms (white); sclerophyll taxa (hatched). The frequencies of pollen of all taxa are shown as percentages of the dry land plant pollen total; each division represents 5% of the pollen sum. Abbreviations for taxa: *Podoc.* = *Podocarpus*; *Arau.* = *Araucaria*; *Urt.* = *Urticaceae/Moraceae*; *El.* = *Elaeocarpaceae*; *Cun.* = *Cunoniaceae*; *Fr.* = *Freycinetaceae*; *Rapan.* = *Rapanea*; *Casuar.* = *Casuarina*; *Euc. comp.* = *Eucalyptus comp.*

the area, certainly as a result of increased precipitation. High values of *Rapanea*, *Cunoniaceae* and *Freycinetaceae* strongly suggest cooler and effectively wetter conditions than those pertaining today. The vegetation was probably a form of cool temperate rain forest (Nanophyll Mossy forest) and the absence of *Nothofagus* from this suggests that it had already become extinct in the region. The slight increase in sclerophyll pollen in the most recent spectrum possibly represents a decrease in effective precipitation towards the present day level. A more detailed interpretation of this zone is impossible at the moment as it is not known whether the pollen sequence is very condensed or whether the mud failed to accumulate through much of the period.

From the results presented, it is evident that climatic changes have occurred in north-east Australia of a magnitude similar to those of glaciated higher latitudes. The main variable, however, has been shown to be rainfall, with the latter part of the Pleistocene effectively drier than the Holocene. The effects of these climatic changes on the vegetation have been dramatic, three major vegetation types having been represented on the Atherton Tableland within the last 60,000 yr.

Work on Lynch's Crater is continuing; recent drilling has extended the recovery of core material beyond 40 m from the surface and still the deepest organic sediments have not been reached. When pollen from the total depth of material has eventually been analysed, the information recovered will provide a record of vegetation and climatic changes occurring through one or several complete glacial cycles and permit an informed assessment of the status of present-day vegetation patterns within the region.

I thank the Australian National University for supporting this work, Messrs A. and K. Anderson for permission

to carry out field work on their property, Professor D. Walker and Dr J. M. Powell for advice and the staff of this department for assistance.

A. P. KERSHAW

Department of Geography,
Monash University,
Clayton, Victoria, Australia

Received April 16; revised June 18, 1974.

¹ Kershaw, A. P., *New Phytol.*, **69**, 785-805 (1970).

² Kershaw, A. P., *New Phytol.*, **70**, 669-681 (1971).

³ Webb, L. J., *Ecology*, **49**, 296-311 (1968).

⁴ Kershaw, A. P., thesis, Australian National University (1973).

⁵ Barbeti, M., and Allen, H., *Nature*, **240**, 46-48 (1972).

Experimental allergic neuritis induced by sensory nerve myelin may provide a model for nonlepromatous leprosy

EXPERIMENTAL allergic neuritis (EAN) is induced by the injection of peripheral nerve myelin and Freund's complete adjuvant into experimental animals¹. It is characterised clinically by an acute motor paralysis accompanied by ataxia, both of which vary in severity from a mild to an extremely severe form. Histology shows focal areas of demyelination and mononuclear cell infiltration confined to the peripheral nervous system (PNS). It is one of a group of autoimmune delayed hypersensitivity conditions which are specific for target tissue but not for species. Experimental allergic encephalitis (EAE) is also an experimentally

induced paralysing disease, but in this case the pathology is confined to the central nervous system (CNS). It is induced by injection of myelin of central nervous system origin in Freund's adjuvant. The observation that myelin from the CNS produced lesions confined to the CNS, whereas that from peripheral nerves produced lesions in the PNS has now been explained on the basis of biochemical differences between myelin from the two sites of origin^{2,3}.

Patients with nonlepromatous (tuberculoid or borderline) leprosy can develop acute sensory loss with selective involvement of superficial sensory modalities⁴. In such patients *Mycobacterium leprae* are few in number. In lepromatous leprosy, although bacteria are abundant, sensory loss occurs late in the disease, at a time when the bacteria have become scarce. This raises the possibility that the sensory loss may be an autoimmune reaction to target sensory nerve supplying the skin. If this is so it should be possible to demonstrate differences in antigenic properties between myelin from peripheral sensory (sural) nerve and that from mixed (sciatic) nerve, which we have previously used to produce EAN⁵. This might prove an experimental model for studying nonlepromatous leprosy. We report here some initial findings after observing for up to nine months, rabbits and rats which were injected with sural nerve in Freund's adjuvant. In all 9 rabbits and in 8 out of 16 rats, skin lesions suggestive of nonlepromatous leprosy were produced.

Human sural nerve obtained *post mortem* was used as antigen. Details of preparation are described elsewhere⁵. In nine Dutch Bantam rabbits 0.2 ml of the suspension was injected into the footpad of one hind limb and 0.05–0.1 ml injected similarly into 16 Sprague Dawley rats. Biopsies of skin lesions were taken from eight rabbits and four rats.

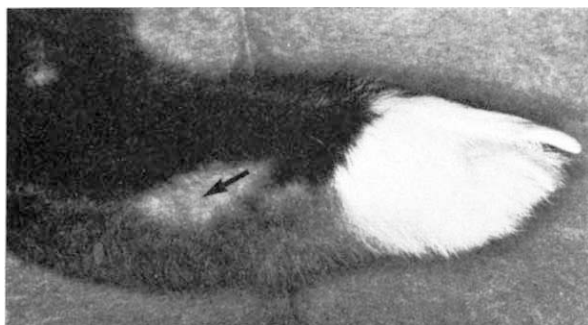


Fig. 1 Skin lesion of 3 week duration on dorsum of rabbit's foot 2 months after injection into same foot pad.

After 4 to 5 weeks the rabbits began to develop discrete skin lesions (Fig. 1) marked by loss of hair without skin ulceration. In some animals with coloured hair, there was a loss of pigment, which was sometimes incomplete, so that patches of hair were light orange, brown, or light grey. The initial lesions were mainly on the injected limbs but later they appeared on the face, other limbs, and dorsum of trunk. Most of the patches were transient, healing after several weeks, but a few have persisted for as long as 3 months. In some the hair began to grow again from the centre. The patches occurred in all nine rabbits, the total number of patches being 30. Some appeared as late as 6 months after injection. Similar denuded patches were observed in the rats. They occurred rather later and were seen in five out of six animals observed for 8 months, and in 10 rats under observations for the past 5 months three have developed patches. The total number was 12. Again some appeared as late as 7 months after injection. None appeared at the injection site. All the limb lesions occurred on the extensor surface, except two which overlapped slightly on to the flexor surface of the hind limb. Only one trunk patch was on the ventral surface. In six rabbits there

was also analgesia to pinprick which was predominantly in the hind limbs and distal in distribution. In rats no convincing analgesia could be demonstrated.

Six of the nine rabbits also developed ataxia, with some motor loss, similar to that seen in EAN, when sciatic nerve was used as antigen source. Two of these animals died. The other four recovered from motor symptoms within 3 weeks. None of the 16 rats, including the eight with skin lesions, showed any ataxia or motor loss. Biopsy of the skin lesions showed erosion and disappearance of hair follicles in the early lesions. In a few there was also granuloma formation with mononuclear cells surrounded by lymphocytes. Dermal nerves showed a mononuclear cell infiltrate.

The clinical picture in the rabbits and rats using myelin from the purely sensory sural nerve as a source of antigen showed distinct differences from that seen when mixed (sciatic) nerve is used. The characteristic skin lesions which we have described have not been reported with sciatic nerve as antigen. Also the ataxia and motor loss which is such a prominent feature of the sciatic group did not occur in any of the rats although it did occur in six of the nine rabbits. In the sensory group the skin lesions appeared later than the classical EAN motor and ataxic signs and often ran a more prolonged course.

On this evidence different types of peripheral nerves may be assumed to differ from one another in antigenic potentiality. Biochemical analysis of the myelin from sural nerve has not been reported, but differences have been reported between sciatic nerve, dorsal roots and brachial plexus in the same species⁶; also the ultrastructural appearance varies slightly between fibres of different sizes in the CNS (ref. 7) and there are variations in the electrophoretic protein pattern of myelin from different parts of the CNS (ref. 8). EAN arising from injection of sciatic nerve has been suggested as a model for Guillain-Barré polyneuritis, a predominantly motor syndrome. The 'sensory' EAN which we have described resembles more closely the clinical and pathological features seen in nonlepromatous leprosy. The outstanding features of both are the discrete skin lesions characterised by lack of pigmentation, depilation, and healing from the centre, with a predilection for the extensor surfaces⁹. Histologically our experimental skin lesions resemble those of early human leprosy¹⁰, with erosion of hair follicles and mononuclear cell infiltration of dermal nerves. In a few of the experimental lesions there was also granuloma formation with mononuclear cells surrounded by lymphocytes.

Skin lesions similar to those in nonlepromatous leprosy have not previously been produced experimentally. The presence of lymphocytes in such skin lesions has been taken as evidence of a cell-mediated immune response directly to *M. leprae*¹¹. But the injection of viable *M. leprae* into mice has failed to reproduce these skin lesions¹². Our experiments suggest instead that *M. leprae* attack the Schwann cell or myelin of sensory peripheral nerves with release of antigen, the immune response of the patient being directed specifically against this antigen. The skin lesions of nonlepromatous leprosy are therefore essentially an autoimmune response to sensory cutaneous peripheral nerve.

C. L. C. is supported by Action Research for the Crippled Child and E. M. E. by the Medical Research Council.

C. L. CRAWFORD
D. H. L. EVANS
E. M. EVANS

Department of Anatomy and Embryology,
University College,
London, WC1E 6BT, UK

Received May 23; revised June 26, 1974.

¹ Waksman, B. H., and Adams, R. D., *J. exp. Med.*, **102**, 213–236 (1955).

- ² Laatsch, R. H., Kies, M. W., Gordon, S., and Alvord, E. C., *J. exp. Med.*, **115**, 777-788 (1962).
- ³ Wolfgram, F., and Kotorii, K., *J. Neurochem.*, **15**, 1291-1295 (1968).
- ⁴ Crawford, C. L., *Lep. Rev.*, **39**, 9-13 (1968).
- ⁵ Allt, G., Evans, E. M., and Evans, D. H. L., *Brain Res.*, **29**, 271-291 (1971).
- ⁶ Greenfield, S., Brostoff, S., Eylar, E. H., and Morell, P., *J. Neurochem.*, **20**, 1207-1216 (1973).
- ⁷ Hildebrand, C., *J. Neurocytol.*, **1**, 223-232 (1973).
- ⁸ Ansari, K. A., and Komanduri, V., *Neurology*, **24**, 94-98 (1974).
- ⁹ Hansen, G. A., and Looft, C., *Leprosy in its Clinical and Pathological Aspects*, 6, 55 (Wright, Bristol, 1973).
- ¹⁰ Ridley, D. S., *J. Path.*, **3**, 191-206 (1973).
- ¹¹ Turk, J. L., *Bull. Wild Hlth Org.*, **41**, 779-792 (1969).
- ¹² Rees, R. J. W., Weddell, A. G. M., Palmer, E., and Pearson, J. H. M., *Br. Med. J.*, **3**, 216-217 (1969).

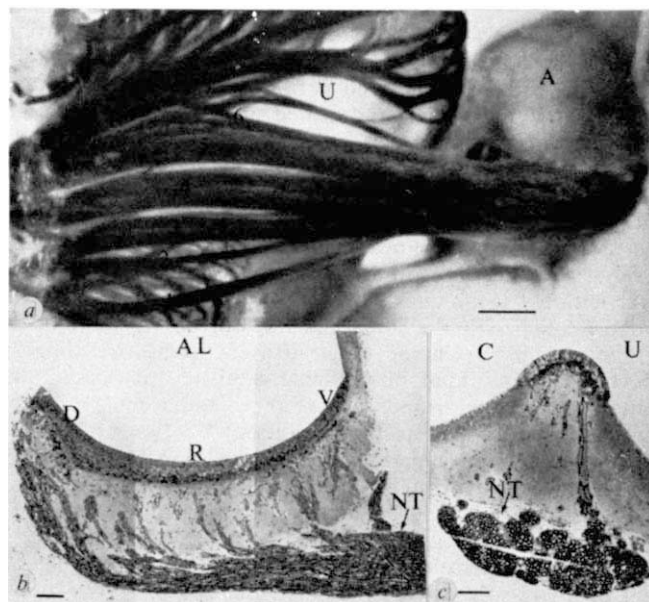


Fig. 1 *a*, Photomicrograph of the right horizontal ampullary nerve innervating the horizontal ampulla (A) of the guitarfish semicircular canal. Intact labyrinth specimens were stained with Sudan black B to facilitate microdissections. The ampullary nerves separate from those of the utricle (U) as individual bundles (numbered in a rostral-caudal sequence) which project dorsally to converge into a nerve trunk (NT) as they approach the ampulla. The nerve trunk courses along the long axis of the crista. The saddle-shaped horizontal crista projects into the ampulla in a medial-lateral plane at nearly right angles to the plane of this figure. The marker equals 0.2 mm. *b*, A longitudinal section through the centre of the left ampullary lumen (AL) and the sensory region, the crista (V, R, D). Labyrinths for histological preparation were fixed *in situ* with glutaraldehyde-paraformaldehyde in 0.1 M phosphate buffer containing 10% sucrose after cervical dislocation and rapid removal of the surrounding cartilage. After embedding in Araldite 502, the tissue was sectioned at 0.5 μ m and stained with aqueous toluidine blue. The plane of this figure is rotated 90° about the long axis of the crista relative to (*a*). Fibres from the nerve trunk (NT) closest to the crista separate and cascade systematically toward the receptor cells, first to the ventral planum semilunatum (V), then along the ridge (R), and finally to the dorsal planum semilunatum (D). The marker equals 0.1 mm. *c*, A cross section of the nerve trunk (NT) and the crista. The plane of this section is perpendicular to the minor axis of the crista and oriented 90° relative to the plane of (*b*). The nerve fibres project from the anterior portion of the nerve trunk toward receptor cells on the slope facing the utricular opening of the semicircular canal (that is, rostrally). The marker equals 0.1 mm.

Functional and anatomical correlation of afferent responses from the isolated semicircular canal

THE semicircular canal is considered classically to be a sensor of angular head motion during animal navigation; however, the functional significance of the canal's morphology and innervation for transduction and encoding of specific head trajectories is unclear. Neural responses from single afferent fibres innervating the crista of isolated elasmobranch labyrinths were used by Groen *et al.* to determine linear system coefficients of an overdamped pendulum equation, considered to reflect directly the dynamics of the motion of the cupula-endolymphatic fluid in the ampulla¹. Similar studies on other preparations revealed certain neural response characteristics that were at variance with simple pendulum models, and these differences were ascribed variously to differences in hair cell physiology and afferent or efferent innervation properties, whose effects were superimposed on the fundamental fluid pendulum dynamics²⁻⁴. But in addition to these hypotheses based on cellular influences, the topological organisation of the receptor could result in specific afferent response characteristics deriving from different anatomical regions within the crista. As evidence for the latter, we report diverse classes of afferent responses, describable by distinctly different linear system equations, that are correlated with the position of separate bundles innervating specific areas of the crista of the guitarfish, *Rhinobatos productus* (Fig. 1).

The horizontal ampullary nerve (HAN) separates from the utricular nerve as six to eight individual bundles which curve and proceed dorsally toward the ampulla (Fig. 1*a*). We counted myelinated fibres in the entire HAN of four preparations and found a mean of 1,359 (range 1,297-1,459) fibres. The individual nerve bundles varied in size, but the rostral-most and caudal-most generally contained fewer fibres (mean: 176, range: 87-367) relative to the central bundles (mean: 288, range: 201-577).

The individual nerve bundles, which appeared to retain their relative spatial distribution, converged near the ampulla, forming a nerve trunk which coursed along the major axis of the saddle-shaped crista (Fig. 6 in ref. 5) from its ventral to its dorsal extremities (Fig. 1*a*). In histological section planes through this major axis (receptor cells and nerves sectioned longitudinally), fibres nearest the crista separated and arched toward the hair cells lining the surface of the ventral planum semilunatum. The remaining fibres cascaded upward progressively along the ridge of the crista and finally projected into the dorsal planum semilunatum (Fig. 1*b*). Hence, fibres from the nerve trunk projected systematically to the crista along its ventral-dorsal axis.

A systematic projection of nerve fibres appeared also across the crista along a minor axis in the plane showing cross sections of both the nerve trunk and the crista (a

rostral-caudal plane, Fig. 1*c*). Nerve fibres from the caudal portion of the nerve trunk were traced in serial sections to hair cells on the canal (caudal) slope, fibres from the central region to the crest of the ridge, and those from the rostral portion to the utricular (rostral) slope. This distribution of fibres was also apparent with a dissecting microscope. On the basis of microdissections and histological preparations, we conclude that a systematic pattern of innervation exists along both the major and minor axes of the horizontal crista.

We recorded single-unit spike trains by suspending severed nerve sub-bundles in air with a forceps electrode¹. Spike trains were amplified, transmitted by means of frequency modulation telemetry, recorded and displayed conventionally. The preparation was aligned on the rotational axis of a Goerz/Inland rotating table (model 800) with the horizontal semicircular canal plane oriented parallel to that of the table.

We developed a new technique to characterise sensory systems on-line by cross-correlating their afferent response

to a white noise pseudorandom binary acceleration stimulus (PRBS)⁸⁻⁹. Cross-correlations of the spike train responses with six to sixteen periods of the PRBS stimulus were computed on-line and scaled appropriately to obtain estimates of the linear system unit impulse response (UIR) for each afferent⁹. The PRBS input afforded significant advantages over the use of conventional deterministic inputs (for example, sinusoidal and constant acceleration): accurate small-signal linear characteristics were determined over wide bandwidths in a fraction of the time required for conventional techniques, and other noise sources that were uncorrelated with the input did not corrupt the cross-correlated results^{7,10}. The accuracy of the UIR as an estimator of the afferent input-output characteristics was tested by observing whether responses to other inputs could be predicted correctly by linear convolution^{9,10}. Conventional inputs were applied experimentally in addition to the PRBS input, and the percentage mean-square-error (% MSE) of the predicted and experimental outputs was computed. Most units showed a sufficiently small % MSE (<15%) to justify using linear estimation for this system.

Our analysis is based on results from 120 cells. We found a pattern of organisation in the UIR characteristics that was correlated with relative position of the parent bundles (Fig. 2). Responses from central bundles, for example, 3

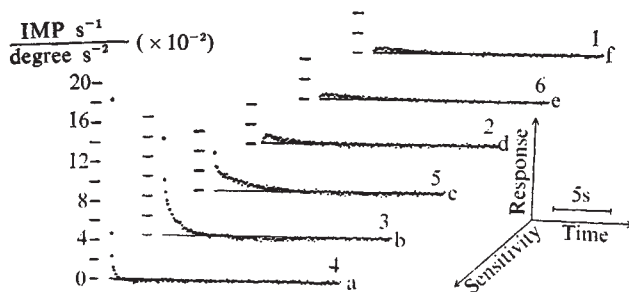


Fig. 2 Linear system impulse response estimates obtained by cross-correlating pseudorandom binary white noise inputs with spike train outputs from six afferents innervating the left horizontal semicircular canal of the guinea-fish. Isolated labyrinths were prepared for afferent response recording by pithing the brain, removing the cranium and cartilage at the rear of the orbit to expose the horizontal ampullary nerve, and mounting the labyrinth on a rotating table. A pseudorandom binary sequence (PRBS) with white noise properties, generated by a PDP-11 computer program, was converted to a sequence of rotational accelerations of magnitude ± 100 degree s^{-2} and direction that varied randomly within each PRBS period. The stimulus pattern was repeated periodically with a period $N\Delta t$ as determined by the duration Δt of individual PRBS states and the total number N of states in each period. The PRBS stimulus has a spectral density that is theoretically constant (within 3 dB), and is by definition a white noise source over the band from $1/N\Delta t$ to $1/3\Delta t$ Hz, approximately. We used values of $\Delta t = 92$ ms and $N = 255$ to result in a period of 23.5 s and a noise bandwidth from 0.04 to 4 Hz. Correction factors were applied to compensate for input transducer error resulting from the rotating table's finite switching time and the PRBS finite bandwidth. The adjacent points are separated by 92 ms intervals. The relative location of the parent bundle of each afferent is indicated in the figure by number for each response beginning at the most rostral bundle in the nerve fan. Responses (a)–(c) are typical of those from bundles located centrally in the nerve fan (numbered 3 and 4) and exhibit an initial peak of varying magnitude (for example, 18.3 in (a) followed by an approximately exponential decay and overshoot of the baseline. Responses (d)–(f) are typical of those from bundles located at the rostral and caudal extremities of the nerve fan and exhibit a resolvable exponential rise followed by an exponential decay with negligible overshoot of the baseline analogous to an overdamped second-order linear system. Sensitivity is defined arbitrarily as the magnitude of the response predicted (by linear convolution) to a step input of magnitude 1 degree s^{-2} and duration 0.46 s, that is, the sum of the amplitudes of the first five points in each impulse response.

and 4 in Fig. 1a, had the form shown in Fig. 2a–c; an initial deflection followed by a decay toward the baseline with a slight overshoot indicative of adaption². In contrast, the responses from the rostral and caudal bundles, for example, 1 and 6 in Fig. 1a, had the contours shown in Fig. 2d–f: a resolvable rising component followed by a slower decay toward the baseline analogous to an overdamped second-order linear system impulse response¹¹. Responses from bundles 2 and 5 were mixed and included all contours shown in Fig. 2. Variability was found in both the relative magnitudes of the initial points and the time constants of the rise and decay components as determined by a nonlinear regression program. The peak amplitudes of all responses from bundles 1 and 6 were similar to those in Fig. 2d–f, indicating lower sensitivities in these afferents relative to those from central bundles. We conclude that the type of response was indeed correlated with relative location of the parent bundles which project to specific anatomical regions of the crista.

We excluded the possibility that the response diversity derived from time-dependent degenerative changes in the isolated preparation. Long term recordings (3–8 h) from cells showing responses typical of all types shown in Fig. 2 never showed changes in afferent response characteristics of individual cells during these periods. Furthermore, in paired cell recordings obtained simultaneously using separate electrode channels, typical rostral-caudal and central bundle responses were recorded simultaneously, both immediately and several hours after exposure of the nerve.

These results showing a functional and anatomical correlation of semicircular canal afferent responses suggest an alternative to the view that the afferents encode direct responses to mechanically simple fluid-cupula displacements. Specifically, more complex transductive mechanisms could result in local filtering of the receptor's physiological inputs for the purpose of enhanced signal detection. As an analogy, ideal receivers for known signals corrupted by noise, such as occur in radar, use bandpass filters matched to a particular signal in the sense that the latter is detected in the presence of Gaussian noise with a theoretically optimum signal-to-noise ratio¹². The filter's impulse response is a time-reversal of the matched signal's waveform. The afferent fibres of the semicircular canal could thus be considered an ensemble of bandpass filters that are matched, in a signal detection theory sense, to specific head acceleration trajectories determined by each afferent's impulse response reversed in time. Each afferent could then be considered tuned to a particular range of head accelerations analogous to the afferent encoding of specific stimulus features described for other sensory systems¹³.

DENNIS P. O'LEARY*
ROBERT F. DUNN
VICENTE HONRUBIA

Department of Surgery, Division of Head and Neck,
University of California, Los Angeles 90024

Received April 16; revised June 24, 1974.

*Present address: Department of Otolaryngology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213.

¹ Groen, J. J., Lowenstein, O., and Vendrik, A. J. H., *J. Physiol. Lond.*, **117**, 329–346 (1952).

² Fernandez, C., and Goldberg, J. M., *J. Neurophysiol.*, **34**, 661–675 (1971).

³ Precht, W., Llinás, R., and Clarke, M., *Expl Brain Res.*, **13**, 378–407 (1971).

⁴ Ledoux, A., *Acta Oto-Rhino-Laryngol. Belg.*, **12**, 113–346 (1958).

⁵ Wersall, J., *Acta Otolaryngol.*, Supp. 126 (1956).

⁶ Briggs, P. A. N., Hammond, P. H., Hughes, M. T. G., and Plumb, G. O., *Proc. Inst. mech. Engng.*, **179**, 37–51 (1965).

⁷ Davies, W. D. T., *System identification for self-adaptive control* (Wiley-Interscience, London, 1970).

⁸ Godfrey, K. R., and Murgatroyd, W., *Proc. Inst. elect. Engng.*, **111**, 1803–1815 (1964).

- ⁹ Lee, Y. W., *Statistical theory of communication* (Wiley, New York, 1960).
- ¹⁰ Marmarelis, P. Z., and Naka, K.-I., *J. Neurophysiol.*, **36**, 605-617 (1973).
- ¹¹ Milsum, J. H., *Biological control systems analysis* (McGraw-Hill, New York, 1966).
- ¹² Whalen, A. D., *Detection of signals in noise* (Academic Press, New York, 1971).
- ¹³ Loewenstein, W. R., *Principles of receptor physiology, handbook of sensory physiology*, **1** (Springer, New York, 1971).

Dissociation of lymphocyte activating determinants from recognition sites in mouse MLR

PREVIOUSLY, we were able to block mixed lymphocyte reactions (MLR) in man using anti-light chain antibody¹ but we have been unable to confirm this observation in subsequent studies using other anti-light chain sera. We have now found that MLR in the mouse is unaffected by a variety of anti-immoglobulin sera but using alloantisera it has become evident that the stimulator cells, but not the responder lymphocytes, can be blocked.

Female mice of 5-6 weeks of age of the following inbred and F₁ hybrid strains were used: BALB/c, C57BL, CBA/H, C3H, BALB/c × CBA/H F₁, BALB/c × C57BL F₁, BALB/c × DBA/2 F₁, CBA/H × AKR/J F₁ and BALB/c × C3H F₁ (breeders obtained from the Laboratory Animals Centre,

Carshalton, Surrey). Rabbit antisera to mouse immunoglobulins were prepared as described elsewhere². BALB/c anti-C57BL and C57BL anti-BALB/c alloantisera were raised by one skin grafting followed by six weekly injections of C57BL and BALB/c spleen cells respectively into male and female recipient mice. One spleen was used for six mice. C57BL anti-BALB/c serum and BALB/c anti-C57BL serum showed 90% cytotoxicity to a titre of 1/100 and 1/80 respectively. Normal BALB/c and C57BL sera as well as all antisera were stored at -40° C.

Foetal calf serum (FCS, Difco, Detroit, Michigan), normal sera and mouse antisera were inactivated for 30 min at 56° C. The normal mouse sera and alloantisera were absorbed for 2 h in a water bath at 28° C, with CBA/H (Tables 1, 2) or syngeneic (Table 3) spleen cells (1 spleen per ml of serum) to eliminate nonspecific inhibitory factor(s). The spleen cells were washed twice with Eagle's medium (MEM, Wellcome, Beckenham, England), before they were used for absorption.

Lymph node suspensions were prepared as previously described^{3,4} except that the cells were finally resuspended in equal ratios of RPMI 1640 (Gibco, Grand Island, New York), and Eagle's medium with 5% FCS.

Parent and F₁ lymphocytes at the concentrations of 2.66×10^6 ml⁻¹ were mixed with 5% absorbed normal serum or appropriate antiserum and then mixed in equal volumes to give a final concentration of 2.5×10^6 cells ml⁻¹. These parent-F₁ MLCs (refs 1 and 5) were set up in 0.5 ml volumes in each E2R culture tube between selected combinations, incompatible for H-2 or M-locus. The sera were allowed to remain in the

Table 1 Specific blocking of stimulating cells in MLC with C57BL anti-BALB/c

Responding cells (H-2; Mls)	Stimulating cells (H-2; Mls)†	¹⁴ C-Thymidine incorporation* on day 3.5 when cultured in presence of:			
		Normal C57BL serum	Index of stimulation	C57BL anti-BALB/c serum	Index of stimulation
C57BL (b;b)	—	823 ± 70		852 ± 88	
—	(C57BL × BALB/c)F ₁ (bd;bb)	63 ± 16		75 ± 21	
C57BL	(C57BL × BALB/c)F ₁	4,991 ± 73	11.3	1,008 ± 154	2.2
—	(C57BL × CBA/H)F ₁ (bk;bb)	1,104 ± 118		322 ± 49	
C57BL	(C57BL × CBA/H)F ₁	7,221 ± 101	9.6	5,195 ± 134	8.8
CBA/H (k;b)	—	900 ± 30		842 ± 43	
—	(CBA/H × AKR/J)F ₁ (kk;ba)	986 ± 70		833 ± 38	
CBA/H	(CBA/H × AKR/J)F ₁	24,206 ± 1,265	25.7	21,633 ± 590	25.8

Normal C57BL and C57BL anti-BALB/c sera were decomplexed, absorbed with CBA/H spleen cells to remove nonspecific inhibitory factor and used at the final dilution of 5%. Experiments performed using C57BL anti-BALB/c serum, decomplexed and absorbed with syngeneic (C57BL) spleen cells showed that the blocking effect is specific to the stimulating cells and absorption of alloantiserum with syngeneic or third party spleen cells makes no difference.

* Expressed as mean c.p.m. ml⁻¹ of triplicates ± s.e. † Mls = M-locus

Table 2 No effect of C57BL anti-BALB/c alloantiserum on responding cells in MLC

Responding cells (H-2; Mls)	Stimulating cells (H-2; Mls)†	¹⁴ C-Thymidine incorporation* on day 3.5 when cultured in presence of:			
		Normal C57BL serum	Index of stimulation	C57BL anti-BALB/c serum	Index of stimulation
BALB/c (d;b)	—	755 ± 52		757 ± 63	
—	(BALB/c × C57BL)F ₁ (db;bb)	869 ± 71		281 ± 13	
BALB/c	(BALB/c × C57BL)F ₁	15,385 ± 788	18.9	13,677 ± 770	26.3
—	(BALB/c × CBA/H)F ₁ (dk;bt)	4,385 ± 126		2,740 ± 270	
BALB/c	(BALB/c × CBA/H)F ₁	11,951 ± 801	4.6	13,356 ± 740	4.9
—	(BALB/c × DBA/2)F ₁ (dd;ba)	3,405 ± 21		2,465 ± 148	
BALB/c	(BALB/c × DBA/2)F ₁	14,823 ± 430	7.1	12,216 ± 436	7.6
CBA/H (k;b)	—	4,316 ± 130		3,107 ± 118	
—	(CBA × AKR/J)F ₁ (kk;ba)	4,390 ± 92		3,554 ± 216	
CBA/H	(CBA/H × AKR/J)F ₁	43,276 ± 371	9.9	39,327 ± 933	11.8

* Expressed as c.p.m. ml⁻¹ of triplicates ± s.e. † Mls = M-locus

Table 3 No effect of BALB/c anti-C57BL alloantiserum on responding cells in MLC with specific blocking of stimulating cells

Responding cells (H-2;Mls)	Stimulating cells (H-2;Mls)†	¹⁴ C-Thymidine incorporation* on day 3.5 when cultured in presence of: Normal BALB/c serum	Index of stimulation	BALB/c anti-C57BL serum	Index of stimulation
BALB/c (d;b)	—	434 ± 28		364 ± 23	
—	BALB/c × C57BL(F ₁ (db;bb))	425 ± 32		489 ± 27	
BALB/c	(BALB/c × C57BL)F ₁	2,226 ± 132	5.3	824 ± 45	1.9
—	(BALB/c × CBA/H)F ₁ (dk;bb)	354 ± 8		381 ± 26	
BALB/c	(BALB/c × CBA/H)F ₁	2,750 ± 176	7.0	2,536 ± 120	6.8
—	(BALB/c × DBA/2)F ₁ (dd;ba)	2,553 ± 67		1,792 ± 73	
BALB/c	(BALB/c × DBA/2)F ₁	4,096 ± 142	2.7	10,357 ± 497	9.6
C57BL (b;b)	—	597 ± 57		1,133 ± 61	
C57BL	(BALB/c × C57BL)F ₁	4,641 ± 376	9.1	8,144 ± 300	10.0

Normal BALB/c and BALB/c anti-C57BL sera were decanted, absorbed with syngeneic BALB/c spleen cells to remove nonspecific inhibitory factor and used at the final dilution of 5%.

* Expressed as c.p.m. ml⁻¹ of triplicates ± s.e. † Mls = M-locus

cultures throughout the experiment. The cultures were refed with equal ratios of Eagle's and RPMI with 5% FCS on day 2. ¹⁴C-Thymidine incorporation was measured on day 3.5 according to the technique previously described^{4,5}. Index of stimulation was calculated as counts in parent plus F₁ MLC divided by mean value of parent and F₁ cultured alone.

Rabbit antisera to immunoglobulin light chains, Fab fragment or the various heavy chain subclasses in no instance inhibited MLR between H-2 incompatible cells; if anything, they were at times responsible for some stimulation (Table 4).

Table 1 shows the results of experiments in which the anti H-2 alloantisera directed against the F₁ stimulator cells are introduced into H-2 complex incompatible parent-F₁ mixed cultures. C57BL lymph node cells were responding to BALB/c × C57BL F₁ target lymph node cells. The antiserum was absorbed with third party (CBA/H) or syngeneic (C57BL) spleen cells. Both methods of absorption were equally effective in removing nonspecific inhibitory activity of mouse serum. The controls were of two kinds—the third party F₁ cell was from BALB/c × CBA/H F₁ lymph nodes and the third party MLC involved an unrelated H-2 compatible, M-locus incompatible combination, that is, CBA/H (H-2^k; Mls^b) and CBA/H × AKR/J F₁ target (H-2^k/H-2^k; Mls^b/Mls^a). The results show that only when the alloantiserum was directed towards the specificity of stimulating cells was there 'blocking' of MLR (the stimulation was reduced from 11.3 to 2.2 times the background). There was no significant reduction of stimulation with any of the controls. Experiments were then done in which the antiserum was specifically directed against the responding cell in MLC. Results of representative experiments are shown in Tables 2 and 3. When anti-H-2^d serum (C57BL anti-BALB/c) was directed against the BALB/c (H-2^d) responding cells in MLC, no blocking was obtained with any of the H-2 or M-locus incompatible combinations (Table 2). Similarly, when another antiserum was used (BALB/c anti-C57BL), blocking of MLC was observed only when

C57BL antigens were present on the stimulating rather than the responding cells.

Our studies therefore do not provide any evidence for the involvement of immunoglobulin determinants (as recognised in the rabbit antisera used) in the recognition of allogeneic cells in mouse MLR. Similar negative findings were obtained in attempts to inhibit T-cell cytotoxicity in the P815 mastocytoma system using the same anti-immunoglobulin reagents (W. V., I. M. R., and D. B. Thomas, unpublished) in agreement with other authors⁶. We have shown, however, that H-2 alloantisera directed against the stimulating cell, but not the responding cell, can specifically block MLR. We cannot be certain which determinants the serum is reacting against since it is likely to contain antibodies against both the H-2K and H-2D specificity as well as the lymphocyte activating determinants. Our first experiments were with sera which had been absorbed with third party cells to remove nonspecific inhibitory factors and while this was unlikely to affect blocking of the stimulating cells, it is possible that antibodies to receptor sites, if present, might have been removed by this procedure. This objection was met by showing that selective blocking of stimulating cells was still obtained after absorption of the nonspecific inhibitor with syngeneic cells.

If antibodies had in fact been raised against the MLR recognition units then they were not of sufficient titre, avidity or specificity to block the reaction. Another interpretation could be that the antisera did in fact react with the receptors of the responding cells but that no inhibition occurred because the blocked receptors were then rapidly shed and new ones synthesised. What these results do show is that the activating determinants are distinct from the receptor sites. In this sense, the results are in conflict with those of Ceppellini⁷ or Thorsby and Solheim⁸ in man but are consistent with other findings (J. Brochier, I.M.R., and H.F., unpublished). A reason for this discrepancy might lie in the concentration of alloantiserum used by the former authors; if the lymphocyte activating

Table 4 Lack of effect of rabbit anti-mouse immunoglobulin sera on the mouse mixed lymphocyte reaction

Experiment	1				2				3		
Parent + F ₁ combination: (H-2;Mls)	CBA + CBA × BALB/c (k;b) + (kd;bb)				CBA + CBA × BALB/c				BALB/c + BALB/c × C3H (d;b) (dk;bc)		
Antiserum added:	NRS	Anti LC(1)	Anti LC(2)	Anti IgM	NRS	Anti Fab(1)	Anti Fab(2)	Anti IgM	NRS	Anti (IgG1 + IgA)	Anti (IgG2a + b)
Lymphocytes											
Control	510	857	940	530	322	172	170	228	708	882	1,000
Parent + F ₁	3,541	6,816	11,439	2,912	1,404	503	769	1,019	1,426	3,312	4,099
Index of stimulation	6.9	7.9	12.2	5.5	4.4	2.9	4.5	4.5	2.0	3.8	4.1

Figures represent ¹⁴C-thymidine incorporation in c.p.m. (mean of triplicates) on day 3.5. Control values were calculated as half the sum of counts in parental and F₁ cells cultured alone. Index of stimulation calculated as counts in parent + F₁ cultures/counts in control.

NRS, Normal rabbit serum; LC, light chain.

determinant and the recognition site are distinct but, nonetheless, present on the same molecule or molecular complex, one could envisage the possibility that higher antibody levels might be required to block the responding cell than to mask the determinant on the stimulating cell.

We thank Drs G. Torrigiani and F. C. Hay for the gift of antisera to mouse immunoglobulins. This work was supported by the Royal Society, Cancer Research Campaign, London Hospital and London Hospital Medical College and the Medical Research Council.

KUDSIA ABBASI
HILLIARD FESTENSTEIN

Tissue Immunology Unit,
London Hospital Medical College,
London, E1, UK

WINSTON VERBI
IVAN M. ROITT

Department of Immunology,
Middlesex Hospital Medical School,
London, W1, UK

Received April 30; revised June 24, 1974.

- ¹ Greaves, M. F., Torrigiani, G., and Roitt, I. M., *Clin. exp. Immun.*, **9**, 313-328 (1971).
- ² Torrigiani, G., *Clin. exp. Immun.*, **11**, 125-135 (1972).
- ³ Festenstein, H., Sachs, J. A., and Oliver, R. T. D., *Proc. Symp. Immunogenet. H-2 System*, 170-177 (Liblice, Prague, 1970, Karger, Basel, 1971).
- ⁴ Abbasi, K., and Festenstein, H., *Eur. J. Immun.*, **3**, 430-435 (1973).
- ⁵ Festenstein, H., *Lancet*, **i**, 182-183 (1968).
- ⁶ Cerottini, J. C., Nordin, A. A., and Brunner, K. T., *J. exp. Med.*, **134**, 553-564 (1971).
- ⁷ Ceppellini, R., in *Progress in Immunology* (edit. by Amos, D. B.), 973-1025 (Academic Press, New York, 1971).
- ⁸ Thorsby, E., and Solheim, B. G., *Transpl. Proc.*, **5**, 1859-1862 (1973).

High frequency of T lineage lymphocytes in nude mouse spleen

CONGENITALLY athymic mice (nude mice) lack the T cell compartment of the lymphoid system¹⁻³ and their spleen has been extensively used as a source of B cells. It has been shown recently, however, that nude mice have a few T cells⁴ and precursors for T cells⁵. These precursors can repopulate a grafted neonatal thymus and differentiate further⁵, migrate to secondary lymphoid organs⁵ and restore some T-cell-dependent immunity⁶.

Although the T precursors probably originate primarily in the bone marrow, the nude mouse spleen might also contain some precursors. Indeed, a variety of treatments renders within a few hours a proportion of nude mouse spleen cells, presumably lymphocytes, susceptible to cytotoxic anti-T lymphocyte antisera⁷. Moreover, it is claimed that cultivation of nude mouse spleen cells on thymic reticuloendothelial cell monolayers also induces an important rise in characteristic T cell reactivities⁸.

When analysed by immunofluorescence, as many as 40% of nude mouse spleen lymphocytes lack detectable membrane immunoglobulin (Ig), (ref. 5 and F.L. and G.E.R., unpublished) a B cell marker⁹. When reacted with fluorescent mouse antibody directed against the θ antigen^{5,10}, a T lymphocyte marker⁹, up to 20% of nude mouse spleen lymphocytes show a faint fluorescence which is specific (cells are stained with anti- θ -C3H reagents, not with anti- θ -AKR) but too weak to be satisfactory. In preliminary experiments the detection of θ was amplified by using in sequence anti- θ antibody and rhodamine-labelled anti-mouse Ig, a procedure which also stains the B

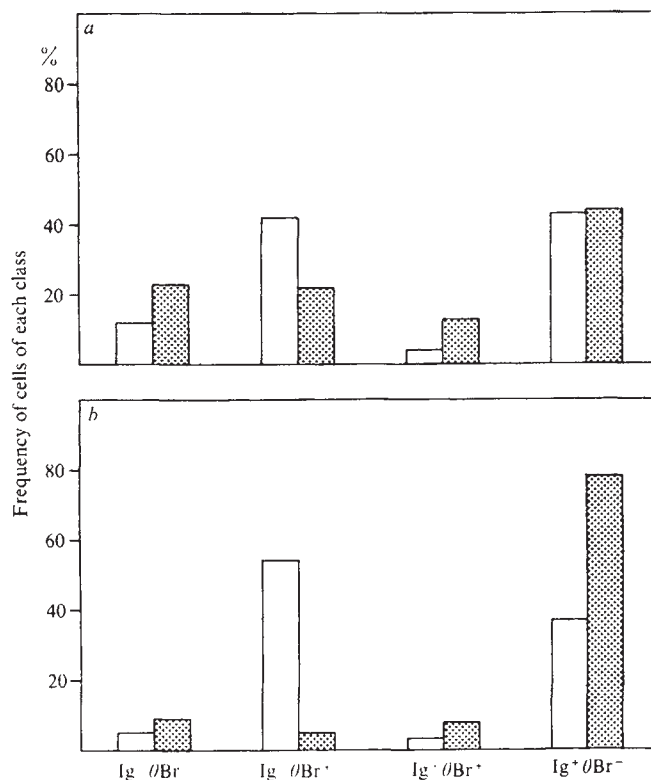


Fig. 1 Frequency of cells with or without membrane Ig and/or θ Br. 2.5×10^6 cells in 0.1 ml medium (RPMI 1640+0.5% BSA+10 mM NaN_3 buffered with 0.03 M HEPES and NaOH to pH 7.2) were incubated (30 min, 0°C) with 0.1 ml (50 μg) of RIG- α -M θ Br (NRIG as control), washed three times, and further incubated (15 min, 0°C) with 0.2 ml of a mixture of TRITC-SIgG- α -RIG (50 μg) and FITC-SIgG- α -MIG (50 μg), washed, and examined for double fluorescence. The figure represents mean values of the frequencies of cells from seven normal mice (five BALB/c, two NMRI) and seven nudes (five BALB/c-nu, two NRM1-nu) (at least 500 cells were examined per individual mouse). *a*, Spleen lymphocytes; *b*, lymph node lymphocytes. Open columns, normal mice; stippled columns, nude mice. For the preparation of RIG- α -M θ Br, immunisation was by two intramuscular injections of 3 CBA/J mouse brain, homogenised in complete Freund's adjuvant at 1 week intervals boosted 5 months later before a wait of 2 weeks and the collection and pooling of immune serum. A pool of preimmune serum from 10 rabbits was used as a control. The isolation of IgG was by repeated $(\text{NH}_4)_2\text{SO}_4$ precipitation (1.6 M, 0°C) followed by DEAE-cellulose chromatography (pH 6.3, 0.0175 M phosphate buffer). The *in vivo* absorption of the non- α - θ antibody in nude mice BALB/c-nu, fourth backcross Gl. Bomholtgård Ltd., Ry, Denmark) was by intraperitoneal injections of 10 mg IgG ml^{-1} per nude mouse, bled out 8-10 h later. The isolation of the RIG from the mouse serum was by the same procedure as above. Most of the mouse Ig (90-95%) sticks to the DEAE, the RIG passes through. The IgG was concentrated on Diaflo membrane UM2 up to 10-20 mg ml^{-1} . Storage was at 4°C , in PBS+0.5% BSA+10 mM NaN_3 . We used RIG- α -M θ Br on cell suspensions at a final concentration of 250 μg ml^{-1} . As a control we used NRIG prepared from the preimmune serum pool and absorbed similarly.

cells through their surface Ig. Calculating the difference between the number of stained cells whether the first step was anti- θ or normal mouse serum, again about 20% of nude mouse spleen lymphocytes seemed definitely to have the θ marker.

To use the amplification provided by indirect fluorescence and at the same time discriminate between B (Ig⁺θ⁻) and T (Ig⁻θ⁺) lymphocytes within a single cell preparation we used the anti- θ antibody present in antisera raised in rabbits against the mouse brain associated θ antigen (θ Br)¹¹ after *in vivo* absorption in nude mice (see legend to Fig. 1).

The basic technique for the detection of membrane markers on viable cells by immunofluorescent reagents in conditions

which do not allow polar redistribution have been described in detail^{5,10,12,13}. Binding of RIgG- α -M0Br was revealed in indirect immunofluorescence using sheep anti-rabbit Ig antibody conjugated with fluoresceine or tetramethylrhodamine (FITC- and TRITC-SIgG- α -RIg)¹². Mouse membrane Ig was detected with FITC- or TRITC-SIgG- α -MIg¹².

When assayed on thymus, spleen and lymph node cells from normal BALB/c and AKR mice (either sex, 8–12 weeks old, Gl. Bomholtgård Ltd., Denmark), the RIgG- α -M0Br stained the expected percentages of T lymphocytes⁹. No significant binding of NRIgG was detected. The specificity of binding for cells of the T lineage was assessed by performing a double labelling of θ and θ Br. AKR or BALB/c mouse cells were first stained by TRITC-C3H IgG- α - θ AKR or TRITC-AKR IgG- α - θ C3H respectively^{5,10}, then reacted with RIgG- α -M0Br (NRIgG as control) which was further detected by FITC-SIgG- α -RIg. The expected proportion of T lymphocytes was labelled in spleen and lymph node cell preparations⁹ and those cells were labelled with both reagents. Moreover, preincubation of BALB/c or AKR lymph node or spleen cells with AKR- α - θ C3H or C3H- α - θ AKR alloantiserum (diluted 1:40, 30 min, 0° C) virtually blocked further binding of the RIgG- α -M0Br. Normal AKR and C3H sera had no inhibitory activity. Thus, the rabbit heteroantibody, although not detectably discriminating between θ C3H and θ AKR, seems to bind to the same or very close membrane determinant(s) than the mouse alloantibody whose specificity was fully assessed earlier^{5,10}.

The binding characteristics of RIgG- α -M0Br are similar to those of α - θ alloantibody: both bind better to thymus lymphocytes than to peripheral T cells, both give a homogeneous binding appearing as a 'ring' fluorescence when detected by fluorescent monovalent Fab- α -Ig. Spots or caps are induced only if a second layer of divalent α -Ig antibody is added.

Double staining for Ig and θ Br was performed. Lymphocytes could be classified into four categories: typical B cells having only membrane Ig (Ig $^{+}$ θ Br $^{-}$), typical T cells having only the θ antigen (Ig $^{-}$ θ Br $^{+}$), cells lacking detectable amounts of both markers (Ig $^{-}$ θ Br $^{-}$) and cells expressing both markers (Ig $^{+}$ θ Br $^{+}$). Normal and nude mice (BALB/c, NMRI, BALB/c-nu and NMRI-nu; Gl. Bomholtgård, Ry, Denmark) were compared (Fig. 1).

In nude mouse spleen the proportion of typical B cells (Ig $^{+}$ θ Br $^{-}$ lymphocytes) is not higher than in normal mouse spleen. Interestingly, about one fifth of the lymphocytes are definitely of T lineage (Ig $^{-}$ θ Br $^{+}$). In lymph nodes typical B cells are more frequent in nudes than in normal mice and many fewer lymphocytes are of T lineage. In both spleen and lymph nodes the frequency of Ig $^{-}$ θ Br $^{-}$ and Ig $^{+}$ θ Br $^{+}$ is higher in nudes than in normal mice.

When spleen cells from a pool of BALB/c-nu were preincubated with AKR- α - θ C3H alloantiserum (diluted 1:40 for 30 min at 0° C) there was an 84% reduction in the number of cells binding detectable amounts of RIgG- α -M0Br (compared with preincubation with normal AKR serum). This shows that, as in normal mice, in nude mice RIgG- α -M0Br also binds apparently to the θ antigen itself and not to an undefined 'stem cell antigen'^{14,15}.

NRIgG did not detectably bind to the cells, except in the case of one NMRI and one NMRI-nu where rare cells picked up NRIgG unspecifically, most of them having also Ig on their surface. These false double positive cells accounted for a quarter of those found when the RIgG- α -M0Br was used on the cells from the same animal.

Ig $^{+}$ θ Br $^{-}$ cells in both normal and nude mice seem to be typical B lymphocytes and do not require further comment but it is interesting that nude mice have a high frequency of lymphocytes of T lineage. The 20% of Ig $^{-}$ θ Br $^{+}$ lymphocytes in nude spleen are not equivalent to those of normal mice; indeed the latter can also be easily detected as θ^{+} by the mouse α - θ alloantibody used in direct immunofluorescence, while

the definite detection of the former depends on the large amplification produced by the indirect immunofluorescence method used. These cells have less θ antigen and it is not known if they are still potential precursors or cells of T lineage having followed an aberrant differentiation pathway because of the lack of thymus.

Ig $^{-}$ θ Br $^{-}$ cells which might be 'early precursors' have already been described^{16,17}, but their possible T nature was rejected¹⁷ on the assumption that the only cells of T lineage homing in the spleen have to come from the thymus.

The significance of Ig $^{+}$ θ Br $^{+}$ cells is difficult to evaluate as it is not known if both markers are the actual synthetic products of the cells.

Cells binding the heavily iodinated synthetic polypeptide (Tyr, Glu)-Ala- -Lys (ref. 18) were found among all four cell types indicating that commitment to antigen and receptor expression on cells of T lineage do not require the thymic presence.

The various cell types described here, and the possibility of a prethymic differentiation pathway of T cells, clearly need further characterisation. It is quite clear, however, that nude mice are not a source of pure B cells, especially when the spleen is used. Conclusions on lymphocyte activation based on this assumption might have to be reconsidered.

We thank Miss L.-B. Hägg, Mrs K. S. Mayor and Miss A. Rydén for technical assistance. One of us (F.L.) is Chercheur Qualifié from the Belgian National Funds for Scientific Research. The sheep antisera were the gift of Dr A. S. Kelus.

FRANCIS LOOR
GEORGES E. ROELANTS

Basel Institute for Immunology,
CH-4058 Basel, Switzerland

Received March 22; revised June 13, 1974.

- ¹ Pantelouris, E. M., *Nature*, **217**, 370–371 (1968).
- ² De Sousa, M. A. B., Parrott, D. M. V., and Pantelouris, E. M., *Clin. exp. Immun.*, **4**, 637–644 (1969).
- ³ Wortis, H. H., *Clin. exp. Immun.*, **8**, 305–317 (1971).
- ⁴ Raff, M. C., *Nature*, **246**, 350–351 (1973).
- ⁵ Loor, F., and Kindred, B., *J. exp. Med.*, **138**, 1044–1055 (1973).
- ⁶ Kindred, B., and Loor, F., *J. exp. Med.*, **139**, 1215–1227 (1974).
- ⁷ Scheid, M. P., *et al.*, *J. exp. Med.*, **138**, 1027–1032 (1973).
- ⁸ Waksal, S. D., Cohen, I. R., Waksal, H. W., and St. Pierre, R. L., *Fedn Proc.*, **33**, 736 (1974).
- ⁹ Raff, M. C., *Transpl. Rev.*, **6**, 52–80 (1971).
- ¹⁰ Roelants, G. E., Forni, L., and Pernis, B., *J. exp. Med.*, **137**, 1060–1077 (1973).
- ¹¹ Golub, E. S., *Cell. Immun.*, **2**, 353–361 (1971).
- ¹² Loor, F., Forni, L., and Pernis, B., *Eur. J. Immun.*, **2**, 203–212 (1972).
- ¹³ Roelants, G. E., Rydén, A., Hägg, L.-B., and Loor, F., *Nature*, **247**, 106–108 (1974).
- ¹⁴ Golub, E. S., *J. exp. Med.*, **136**, 369–380 (1972).
- ¹⁵ Rodt, H., Thierfelder, S., and Eulitz, M., *Eur. J. Immun.*, **4**, 25–29 (1974).
- ¹⁶ Lamelin, J.-P., Lisowska-Bernstein, B., Matter, A., Ryser, J. E., and Vassalli, P., *J. exp. Med.*, **136**, 984–1007 (1972).
- ¹⁷ Stobo, J. D., Rosenthal, A. S., and Paul, W. E., *J. exp. Med.*, **138**, 71–87 (1973).
- ¹⁸ Roelants, G. E., and Rydén, A., *Nature*, **247**, 104–106 (1974).

Restriction by H-2 gene complex of transfer of cell-mediated immunity to *Listeria monocytogenes*

RESULTS from three distinct experimental systems indicate that, except for lymphocytes reacting to alloantigens, interaction between sensitised thymus-derived lymphocytes (T cells) and other somatic cells occurs only in syngeneic or semiallogeneic systems. First, histoincompatible T cells

do not function as helpers for antibody-forming cell precursors (B cells)^{1,2}. Second, maximal proliferation of lymphocytes (presumably T cells) resulting from exposure *in vitro* of sensitised guinea pig lymphocytes to antigen-pulse macrophages is observed only when the two cell types share major histocompatibility antigens³. Third, cell-mediated lysis of fibroblasts or macrophages infected with lymphocytic choriomeningitis (LCM) virus, a function of specifically sensitised T cells, occurs only when lymphocyte and target cells share at least one set of H-2 antigenic specificities^{4,5}. The phenomenon is unaffected by differences either in minor histocompatibility antigens, or at the M locus.

Can this restriction be applied more generally as an index of T cell involvement in immunological phenomena? This possibility can be further investigated in another model where T cells are known to play a central part; adoptive transfer of immunity to *Listeria monocytogenes* infection in mice⁶⁻⁸. Experiments reported here indicate that T cell activity in this experimental system is also restricted by the H-2 gene complex.

The strain of *Listeria monocytogenes*, and methods used for immunisation, cell preparation, cell transfer and enumeration of viable bacteria in individual organs have been described elsewhere^{7,9,10}. In most experiments 5.0×10^7 CBA/H $\times$ C57BL F₁ 6 d *Listeria* immune or normal cells, were injected intravenously (i.v.) into CBA/H or BALB/c recipients, infected i.v. 2 h previously with 5×10^3 viable *Listeria*. Protective effects were measured quantitatively by determining viable bacterial counts in individual recipient spleens and livers at intervals after cell transfer.

Growth kinetics of *Listeria* in CBA/H and BALB/c mice were comparable (controls, Fig. 1). Immune CBA $\times$ C57BL F₁ cells protected semiallogeneic parental CBA/H much more effectively than allogeneic BALB/c cells (Fig. 1). Viable bacterial counts in spleen were a more sensitive indicator of protection than liver counts. Therefore although liver counts were also determined, only viable counts in spleen will be presented, the results for liver being essentially similar. Transfer of normal cells into infected semiallogeneic or allogeneic recipients had no significant influence on bacterial counts in spleen or liver during the first 2 d. In the following experiments recipient mice were killed 42 to 48 h after cell transfer^{7,8,10}.

The degree of protection of CBA/H or BALB/c mice by adoptively transferred immune CBA/H $\times$ C57BL F₁

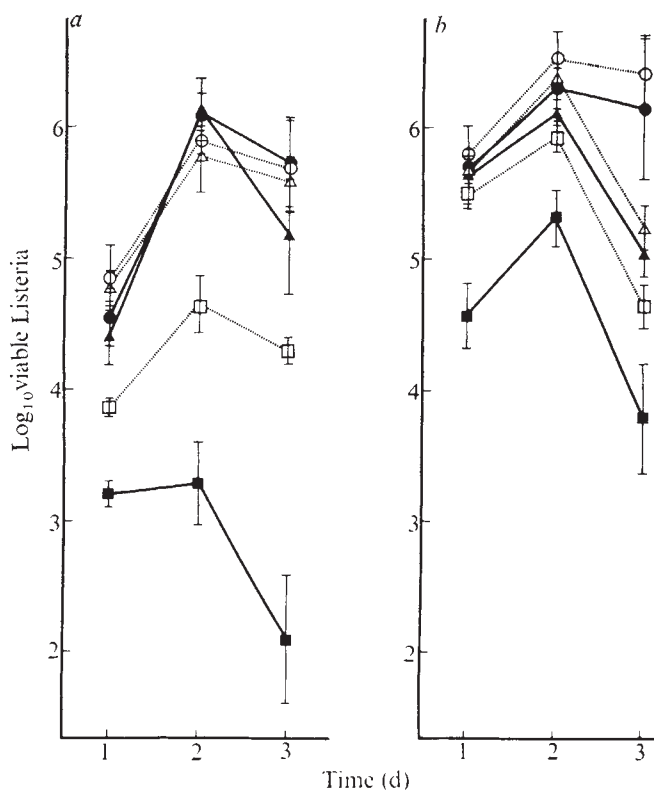


Fig. 1 Effect of the transfer of 5×10^7 immune (■, □) or normal (▲, △) spleen cells (CBA/H $\times$ C57BL F₁) as compared with controls (●, ○) on the viable counts of *Listeria monocytogenes* in a, spleens and b, livers of parental strain CBA/H (■, ▲) or allogeneic BALB/c (□, △, ○) recipients infected with 5×10^3 viable bacteria 2 h before cell transfer. Each point represents the mean of groups of four to five mice. The vertical bars enclose two standard errors of the mean. Control mice and normal cell recipient did not differ significantly, except liver counts on day 3: CBA/H $P < 0.05$, BALB/c $P < 0.01$. Protection by immune cells was significant when compared with no cells or normal cells in CBA/H spleens: day 1: $P < 0.01$; day 2: $P < 0.001$; day 3: $P < 0.01$; livers: day 1: $P < 0.02$; day 2: $P < 0.02$; day 3: $P < 0.05$. For BALB/c spleens: day 1: $P < 0.01$; day 2: $P < 0.02$; day 3: $P < 0.02$; livers: day 1: $P < 0.05$; day 2: $P < 0.05$; day 3: $P < 0.05$. Difference of protection in CBA/H and BALB/c: spleen: day 1: $P < 0.01$; day 2: $P < 0.01$; day 3: $P < 0.001$; liver: day 1: $P < 0.05$; day 2: $P < 0.05$; day 3: $P < 0.05$.

Table 1 Protective effect of transferred immune cells on syngeneic, semiallogenic, and allogenic recipients

Donors (H-2 type)	Recipients (H-2 type)	Log ₁₀ viable bacteria counts		Protection
		immune cells*	normal cells†	
Experiment 1				
CBA/H × C57BL F ₁ (k/b)	CBA/H × C57BL F ₁ (k/b)	3.12 ± 0.21§	5.71 ± 0.15	2.59
CBA/H × C57BL F ₁ (k/b)	CBA/H (k)	3.31 ± 0.27§	6.10 ± 0.19	2.80
CBA/H × C57BL F ₁ (k/b)	C57BL (b)	1.97 ± 0.15§	5.03 ± 0.10	3.06
CBA/H × C57BL F ₁ (k/b)	BALB/c (d)	4.64 ± 0.20 ⁺	5.45 ± 0.20	0.81
Experiment 2				
CBA/H (k)	CBA/H (k)	3.43 ± 0.15§	5.28 ± 0.07	1.85
CBA/H (k)	CBA/H × C57BL (k/b)	3.43 ± 0.11§	5.49 ± 0.11	2.06
CBA/H (k)	BALB/c (d)	4.72 ± 0.15‡	5.30 ± 0.06	0.58¶
CBA/H (k)	C57BL × BALB/c F ₁ (b/d)	4.39 ± 0.18§	5.28 ± 0.07	0.89¶
BALB/c (d)	BALB/c (d)	3.63 ± 0.19§	5.49 ± 0.11	1.86
BALB/c (d)	CBA/H (k)	4.40 ± 0.09‡	5.32 ± 0.13	0.92¶
Experiment 3				
DBA/2 (d)	DBA/2 (d)	4.64 ± 0.23§	6.10 ± 0.09	1.46
DBA/2 (d)	BALB/c (d)	4.37 ± 0.21‡	5.99 ± 0.15	1.62

* Mean $\pm$ s.e.m. of groups of five mice; significance determined with Student's *t* test against normal cells.

† $P < 0.05$

‡ $P < 0.01$

§ $P < 0.001$

¶ Controls (no cells); experiment 1 CBA/H 6.10 ± 0.12 , BALB/c 5.89 ± 0.11 , F₁ 6.13 ± 0.15 ; experiment 2 CBA/H 5.39 ± 0.19 , BALB/c 5.68 ± 0.17 , F₁ 5.50 ± 0.18 .

¶ Significance of protection in syngeneic or semi-allogeneic against allogeneic recipients $P < 0.01$.

‡ 5×10^7 viable normal or immune spleen cells were transferred to recipients. Immune cells were from donors injected with $2-3 \times 10^3$ viable bacteria 6 d previously. Recipients were infected with 5×10^3 viable bacteria 2 h before cell transfer. Log₁₀ viable bacteria were counted in spleens of recipients 42 h after cell transfer.

spleen cells and the restriction of their efficiency to the semiallogeneic donor-recipient combination are clearly dose dependent (Fig. 2). Parental CBA/H mice were protected by 1.7×10^7 or more immune F_1 cells ($P < 0.001$), but not by 6×10^6 cells. BALB/c recipients were only protected by 5×10^7 ($P < 0.01$) or more allogeneic immune cells, but to a lesser extent than CBA/H. A very high cell dose, 1.5×10^8 immune cells, was obviously not much more efficient than 5×10^7 cells in the semiallogeneic host, whereas BALB/c were better protected. The two dose-response relationships, plotted on a log/log basis, were linear and parallel over certain dose ranges (Fig. 2). The efficiency with which the protection is transferred into semiallogeneic CBA/H and allogeneic BALB/c could therefore be compared and differs by a factor of about 4.

Under the chosen conditions 5×10^7 transferred immune cells caused comparable protection in syngeneic or semiallogeneic (F_1 into parent or parent into F_1) hosts and revealed marked restriction of the protective effects in allogeneic recipients (Table 1). As the viable counts in the spleens of control (no cells) mice and of normal cell recipients did not differ significantly in the various strains (except for C57BL), the protective effect of the immune cells could be readily compared.

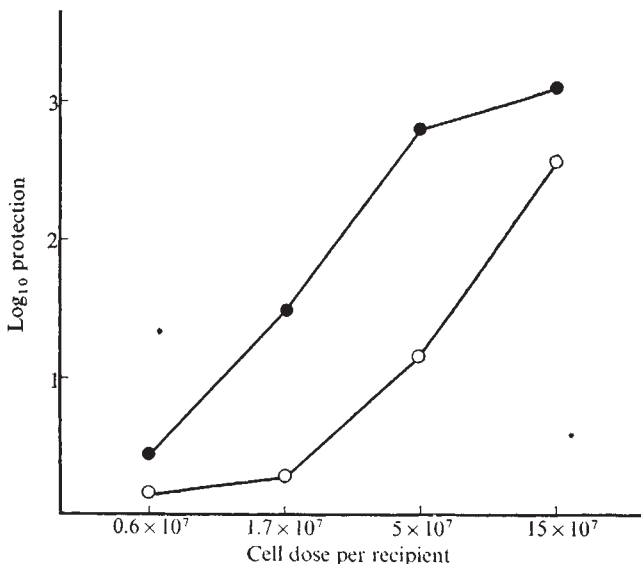


Fig. 2 Protection against *Listeria monocytogenes* by the transfer of various doses of immune spleen cells (CBA/H × C57BL F_1) as compared, with normal cells, into parental strain CBA/H (●) or allogeneic BALB/c (○) recipients, determined as the difference of the mean of log₁₀ viable counts in the spleens of groups of four to five mice. Significance of difference: 0.6×10^7 cells: n.s.; 1.7×10^7 cells: $P < 0.02$; 5×10^7 cells: $P < 0.01$; 1.5×10^8 cells: n.s.

The possibility that a difference at the M locus is responsible for this restriction was investigated by transferring DBA/2 *Listeria*-immune spleen cells into DBA/2 and BALB/c recipients. Both strains share H-2^d antigenic specificities¹¹, but differ at the M locus (Table 1, experiment 3). Incompatibility at the M locus had no obvious effect.

How can the restriction be explained? As only 40 to 48 h elapse between cell transfer and determination of viable bacterial counts, graft versus host (GvH) reactions are unlikely to influence adoptively transferred cells. Also the donor recipient combination CBA/H × C57BL F_1 into CBA/H or BALB/c should give comparable conditions for GvH reaction and homing of transferred cells in both recipient strains. Furthermore, cytotoxic T cells from mice infected with LCM virus home in comparable numbers to the spleens of LCM infected syngeneic or allogeneic

recipients on day 1 after cell transfer (unpublished data).

Events leading to protection against *L. monocytogenes* in the recipients of transferred immune cells can be summarised as follows: *Listeria* organisms injected i.v. are taken up by fixed recipient macrophages⁹. *Listeria* antigens would thus be present in lesions either as free bacterial antigens or associated with living or dead macrophages. Sensitised donor lymphocytes injected later are presumably triggered on contact with the antigen and then probably produce soluble factor(s)^{12,13}, which activate macrophages to the increased bactericidal activity responsible for protection⁹. Donor macrophages play no significant role in protection⁶⁻⁸.

There are potentially two points in this sequence of events at which restriction by the H-2 gene complex could operate.

First, it is possible that the activation of macrophages by a soluble factor only occurs efficiently when macrophages and factor are from the same mouse strain. There is no strict precedent for such a phenomenon, but the species specificity of interferon^{14,15} and mouse migration inhibition factor (MIF)¹⁶ could be a similar phenomenon. This possibility could be readily tested in systems where macrophage activation is achieved *in vitro*^{12,13}.

Second, as antigens presented on the surface of macrophages trigger T cells more efficiently than soluble antigen^{3,4}, the alternative possibility is that antigen presented by histoincompatible macrophages fails to trigger T cells efficiently³. Both possibilities are compatible with the observation that immune allogeneic cells can protect a host if sufficiently large doses of cells are transferred.

Similar mechanisms have been proposed in other systems. For antigen-induced dose-dependent proliferation of primed lymphocytes by antigen-pulsed macrophages to occur, both cell populations must share histocompatibility antigens³. When soluble antigen is added in excess, the restriction by histocompatibility antigens is overcome³. In contrast a secondary *in vitro* anti-DNP response of mouse spleen cells could be triggered equally well by syngeneic and allogeneic macrophages pulsed with DNP-conjugate¹⁷. This could be explained by excess T helper activity being present in the system, thus covering up differences in the efficiency of antigen presentation by histocompatible or incompatible macrophages^{3,18}.

In conclusion, these results further favour the idea that mouse T cell functions in general may be restricted by the H-2 gene complex.

I thank Drs R. V. Blanden and P. C. Doherty for advice and criticism, Miss Gail Essery for technical assistance and the Schweizerische Stiftung fuer Biologisch-Medizinische Stipendien for financial support.

R. M. ZINKERNAGEL

Department of Microbiology,
The John Curtin School of Medical Research,
The Australian National University,
Canberra, Australia

Received May 13, 1974.

¹ Kindred, B., and Shreffler, D. C., *J. Immunol.*, **109**, 940 (1972).

² Katz, D. H., Hamaoka, T., and Benecerraf, B., *J. exp. Med.*, **137**, 1405 (1973).

³ Rosenthal, A. S., and Shevach, E. M., *J. exp. Med.*, **138**, 1194 (1973).

⁴ Doherty, P. C., and Zinkernagel, R. M., *Transplant. Rev.*, **18** (in the press).

⁵ Zinkernagel, R. M., and Doherty, P. C., *Nature*, **248**, 701 (1974).

⁶ Lane, F. C., and Unanue, E. R., *J. exp. Med.*, **135**, 1104 (1972).

⁷ Blanden, R. V., and Langman, R. E., *Scand. J. Immunol.*, **1**, 379 (1972).

⁸ North, R. J., *J. exp. Med.*, **138**, 342 (1973).

⁹ Mackaness, G. B., *J. exp. Med.*, **116**, 381 (1962).

¹⁰ Mackaness, G. B., *J. exp. Med.*, **129**, 973 (1969).

¹¹ Festenstein, H., *Transplant. Rev.*, **15**, 62 (1973).

- ¹² Krahenbuhl, J. L., and Remington, J. S., *J. infect. Immun.*, **4**, 337 (1971).
¹³ Jones, T., and Youmans, G. P., *Cell. Immun.*, **9**, 353 (1973).
¹⁴ Merigan, T. C., *Science*, **145**, 811 (1964).
¹⁵ Baron, S., Babar, S., and Buckler, C. E., *Science*, **145**, 819 (1969).
¹⁶ Youngner, J. S., and Salvin, S. B., *J. Immun.*, **111**, 1914 (1973).
¹⁷ Katz, D. H., and Unanue, E. R., *J. exp. Med.*, **127**, 967 (1972).
¹⁸ Kapp, J. A., Pierce, C. W., and Banecerraf, B., *J. exp. Med.*, **138**, 1121 (1973).

Reactivity of steroid-resistant neonatal thymocytes

THE reactivity of thymus-derived (T) lymphocytes when stimulated with the phytohemagglutinin (PHA), concanavalin A (con A) and pokeweed mitogen (PWM), correlates well with immunological reactivity. Consequently, the mitogens have been useful in studies of the functional maturity of thymocytes in foetal and neonatal mice. Reactivity to con A and PWM increases greatly during the perinatal period, but PHA responsiveness reaches a peak shortly before birth and then declines³. This decline could be due to either (1) reversible suppression of PHA-responsive thymocytes by PHA-unresponsive cells; or (2) the emigration or death of PHA-reactive cells or the immigration or expansion of a population of PHA-unresponsive cells. If PHA-responsive thymocytes in the newborn mouse are all steroid resistant, as seems to be the case in the adult^{4,6}, then steroid treatment of newborns might aid the choice between possibilities (1) and (2). If (1) is correct, steroid treatment should cause a greater increment in thymocyte PHA responsiveness than could be explained by the increased relative number of highly reactive steroid-resistant cells. If (2) is correct, steroid treatment should increase PHA-responsiveness in proportion to the increase in relative number of PHA-responsive cells. If, however, all PHA-responsive cells are eliminated, steroid treatment should not increase PHA responsiveness. This possibility is given some support by a report⁷ involving techniques different from ours. We have therefore attempted to characterise the fraction of thymocytes surviving steroid treatment in the newborn mouse with regard to mitogen reactivity, reactivity in the mixed lymphocyte reaction (MLR), and surface representation of the θ alloantigen. We found that steroid treatment increased the PHA responsiveness of newborn thymocytes, supporting explanation (2).

BALB/c mice about 24 h old were given 100 mg kg⁻¹ hydrocortisone acetate intraperitoneally 48 h before death, together with colloidal carbon to mark parathymic lymph nodes, which were discarded. Controls were given only colloidal carbon. Thymocytes were cultured at 2×10^6 ml⁻¹ in microtitre trays for 72 h in RPMI 1640 supplemented with 5% foetal calf serum, 2 mM glutamine, 15 mM HEPES buffer, and 50 μ g ml⁻¹ gentamicin. PHA-P was used at a final concentration of 1 μ g ml⁻¹; con A was used at 2 μ g ml⁻¹; and PWM at 3 μ g ml⁻¹. Allogeneic target cells were prepared by incubating C57BL/6 spleen cells for 30 min at 37°C in 40 μ g ml⁻¹ mitomycin C, followed by three

washes in Hanks balanced salt solution (BSS). One microcurie of ³H-thymidine (specific activity 6.7 Ci mmol⁻¹) was added to each culture well 16 h before termination. Cultures were collected and ³H-thymidine incorporation into trichloroacetic acid-precipitable material was measured in a scintillation counter.

Steroid-treated newborn and adult mice yielded about 16% of the number of thymocytes obtained from untreated controls. Table 1 shows a comparison of the mitogen responses of newborn and adult normal and steroid-resistant thymocytes. Steroid-resistant newborn thymocytes had substantially more PHA reactivity than normal newborn thymocytes. This was true if data were expressed as c.p.m. or as the ratio of experimental to control. The increment in reactivity, however, was no more than would be anticipated if steroid-resistant thymocytes were the only PHA-reactive cells in the newborn thymus, that is, the steroid-resistant fraction, constituting about one-sixth of all thymus cells, responded six times as well as unfractionated thymocytes. This suggests that diminished PHA reactivity in the neonate is not due to strong reversible suppression by steroid-sensitive thymocytes of the PHA-reactive subpopulation, nor to the absence of PHA-reactive cells due to emigration or cell death.

Reactivity of newborn steroid-resistant thymocytes to con A and PWM was substantially greater than that of normal newborn thymocytes. As in the case of PHA, the steroid-resistant subpopulation could account for all the con A and PWM-reactive newborn thymocytes.

In the adult, steroid-resistant thymocytes had much greater PHA, con A and PWM-reactivity than normal thymocytes. As in the newborn, con A and PWM-reactivity in the adult thymus could be accounted for by the steroid-resistant fraction; the steroid-resistant thymocytes were even more reactive to PHA than would be expected. These experiments show that there are comparable numbers of mitogen-reactive cells in the newborn and adult thymus after steroid treatment. If mitogen responsiveness is used as a marker of T cell maturation, then the newborn thymus, like the adult, contains a small pool of functionally mature T cells.

In both newborns and adults, steroid treatment resulted in only a small increment in reactivity to alloantigen (C67BL/6) spleen cells, confirming earlier reports⁸. This may reflect a paucity of MLR-reactive cells in the thymus, or it may be due to a requirement for more than one effector cell type in the MLR.

The ability of thymocytes surviving steroid treatment to absorb the cytotoxic activity of AKR anti- θ C3H serum was used as an index of the relative amount of θ alloantigen expressed on the surface of the cell population. A constant number of normal or steroid-resistant thymocytes was incubated with a 1:4 dilution of high titre anti- θ serum for 30 min at 4°C. Cells were pelleted by centrifugation, and the residual titre of the supernatant (compared with a control tube containing no cells) assayed against ⁵¹Cr-labelled adult BALB/c thymocytes as before⁷. Absorption with adult normal thymocytes markedly reduced the cytotoxic titre (Fig. 1) of the anti- θ serum, whereas adult steroid-resistant cells caused only a small reduction. This confirms reports⁸

Table 1 Mitogen responses of newborn and adult steroid-resistant thymocytes

	No mitogen	PHA	Con A	PWM
Newborn thymocytes	1,099 $\pm$ 239	2,203 $\pm$ 181	5,509 $\pm$ 671	5,885 $\pm$ 593
Newborn steroid-resistant thymocytes*	963 $\pm$ 157	17,406 $\pm$ 1,763	35,256 $\pm$ 6,535	28,265 $\pm$ 3,336
Adult thymocytes	263 $\pm$ 38	1,336 $\pm$ 92	18,837 $\pm$ 1,353	4,201 $\pm$ 1,067
Adult steroid-resistant thymocytes*	581 $\pm$ 285	27,200 $\pm$ 2,296	28,389 $\pm$ 3,994	21,792 $\pm$ 2,204

Numbers represent the means of c.p.m. $\pm$ s.e. of replicates of four per group in one representative experiment. Cells were cultured for 72 h with ³H-thymidine added for the final 16 h of culture.

* Newborn or adult BALB/c mice received 100 mg kg⁻¹ hydrocortisone intraperitoneally 48 h before removal of thymuses for culture.

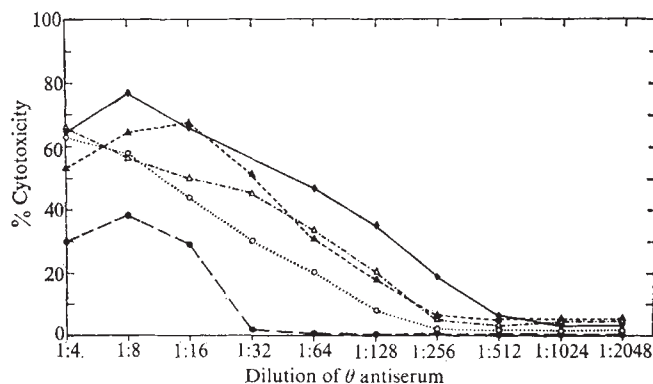


Fig. 1 Cytotoxicity curve for anti- θ C3H serum absorbed with different thymocyte populations and then titred against ^{51}Cr -labelled adult thymocytes. ○, Newborn thymocytes; △, newborn steroid-resistant thymocytes; ●, adult thymocytes; ▲, adult steroid-resistant thymocytes; ◆, unabsorbed anti- θ (C3H).

that normal thymocytes express many θ determinants per cell, while the steroid-resistant subpopulation expresses on the average many fewer θ determinants per cell.

In contrast, however, newborn BALB/c thymocytes (mice <24 h old) from normal mice reduced the antiserum titre much less than comparable numbers of adult thymocytes. Moreover, steroid treatment caused only a marginal reduction in the ability to absorb anti- θ activity. We conclude that newborn thymocytes express somewhat fewer θ determinants per cell than do adult thymocytes, and that, consequently, steroid treatment leads to a smaller shift in the relative θ expression of the surviving subpopulation.

Steroid treatment had similar effects on the morphology of newborn and adult thymus, almost obliterating the thymic cortex. In newborns, however, we noted a few collections of small lymphocytes in the subcapsular region.

We conclude that the potential decline in PHA responsiveness is not due to the selective disappearance from the thymus of PHA-responsive steroid-resistant lymphocytes. On the contrary, all the PHA reactivity of the neonatal thymus can be accounted for by the steroid-resistant cells. It thus seems unlikely that there are many PHA-reactive cells in the steroid-sensitive fraction comprising 80%–90% of thymocytes. Our studies also suggest that the perinatal decline is not due to suppression of PHA-reactive cells by steroid-sensitive thymocytes, for the abolition of such suppression by steroid treatment should have led to a greater increase in PHA reactivity than we observed. The decline in PHA responsiveness, then, could be due to dilution of PHA-reactive cells by PHA-unresponsive cells, or to the emigration of PHA-reactive cells from the thymus. We cannot distinguish between these possibilities although significant numbers of PHA-reactive cells do not appear in the neonatal spleen until 6 or 7 d after the sudden decline in thymic PHA-responsiveness, arguing against emigration to the spleen. Emigration to lymph nodes is a possibility that has not been investigated.

The finding that poorly-reactive normal neonatal thymocytes and highly-reactive steroid-resistant neonatal thymocytes appeared to express relatively similar amounts of surface θ alloantigen suggests that in this instance θ does not serve as a marker for cell differentiation, as proposed for the adult thymus⁹.

Finally, these data emphasise the relative maturity, at least in terms of mitogen reactivity, of cells in the thymus of newborn mice.

We thank Dr William Paul for helpful discussions.

PHILIP L. COHEN

DONALD E. MOSIER

Laboratory of Immunology,
National Institute of Allergy and Infectious Diseases,
Bethesda, Maryland 20014

Received May 20, 1974.

- ¹ Miller, J. F. A. P., and Mitchell, G. F., *Transplant Rev.*, **1**, 3 (1969).
- ² Claman, H. N., and Chaperon, E. A., *Transplant Rev.*, **1**, 92 (1969).
- ³ Mosier, D. E., *J. Immun.*, **112**, 305 (1974).
- ⁴ Blomgren, H., and Andersson, B., *Expl Cell Res.*, **57**, 185 (1969).
- ⁵ Howe, M. L., and Manziello, B., *J. Immun.*, **109**, 534 (1972).
- ⁶ Blomgren, H., and Svedmyr, E., *Cell. Immun.*, **2**, 285 (1971).
- ⁷ Mosier, D. E., and Pierce, C. W., *J. exp. Med.*, **136**, 1484 (1972).
- ⁸ Boyse, E. A., Miyazawa, M., Aoki, T., Old, L. J., *Proc. R. Soc.*, **B170**, 175 (1968).
- ⁹ Owen, J. J. T., and Raff, M. C., *J. exp. Med.*, **132**, 1216 (1970).

Dissociation of EEG and behavioural effects of ethanol provide evidence for a noncholinergic basis of intoxication

IN 1957 it was reported that physostigmine, a drug that potentiates brain acetylcholine by inhibiting the enzyme which normally destroys it, could partially antagonise a tranquilliser, such as chlorpromazine¹. Many years elapsed before anyone tested physostigmine for possible antagonistic effects against other sedative type drugs, such as ethanol. Physostigmine given before but not after massive doses of ethanol (4.5 g kg⁻¹) did seem to shorten sleeping time and to reduce death of mice, an observation that led to the conclusion that ethanol might inhibit acetylcholine release or function^{2,3}.

I thought that this question ought to be examined using more specific measures of ethanol action, in particular the electroencephalogram (EEG) and a battery of behavioural tests for intoxication. Such studies revealed that physostigmine blocked ethanol-induced EEG deactivation (changes to high voltage, slower frequencies) but did not prevent behavioural signs of intoxication. This to my knowledge is a rare demonstration of dissociation of the EEG and behavioural effects of ethanol, although similar dissociations have been reported for reserpine⁴, immobility reflex ('animal hypnosis')^{5,6}, human hypnosis^{7,8}, paradoxical or dream stage of sleep⁹, and certain comatose disease states of dogs¹⁰⁻¹² and humans¹³. The most significant aspect of the ethanol dissociation is that it suggests that intoxication does not primarily involve impairment of cholinergic systems.

EEG recording was performed on eight rats, with chronically implanted epidural electrodes bilaterally placed over frontal, parietal, and occipital cortex. During recording, rats were free to move within a 25 cm² box.

Four standard 'catalepsy' tests were used in a separate study of behaviour. In one test, a 'cork test', the rat was placed on four corks of diameter 3½ cm, one foot on each, with corks spread 8 cm apart laterally and 10 cm apart longitudinally; catalepsy was scored if feet were not moved for 10 s. Two 'platform' tests were used in which both front paws were placed on an elevated platform, either 3 cm high or 9 cm high; catalepsy was defined as holding that position for 10 s. Finally, a 'net' test was used in which the rat was placed on a vertically positioned hardware cloth (5 mm square), 25 cm above the floor; catalepsy was defined as staying on the net for 15 s.

All drugs were administered intraperitoneally in the following doses: physostigmine salicylate (eserine), 0.2 mg kg⁻¹ (a 'teeth chattering' dose); neostigmine methylsulphate (0.2 and 0.3 mg kg⁻¹) (the latter caused collapse and was near the fatal limit); and ethanol, 20% v/v aqueous solution, 1.5 and 2 g kg⁻¹ (both were intoxicating, with the latter causing the most pronounced EEG signs).

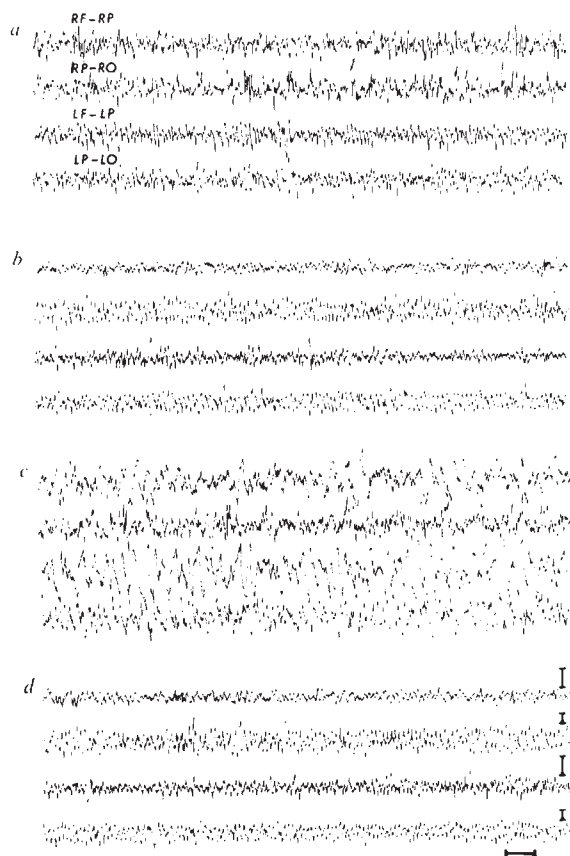


Fig. 1 Representative illustrations of the EEG phenomena exhibited by all rats. All records are from the same freely behaving rat, with 3–4 d between successive trials. *a*, Control records indicate a mildly activated EEG. *b*, Eserine (2 mg kg^{-1}) caused a marked activation (note 6–7 s^{-1} activity) which began 4 min after injection and lasted the full 15 min recording session. *c*, Alcohol (2 mg kg^{-1}) caused pronounced deactivation, beginning 3 min after injection and persisting throughout the recording period. *d*, Administration of eserine 5 min before alcohol injection blocked alcohol-induced deactivation, with EEG activation quite evident for the full 15 min. Behavioural tests of this rat and the others during this eserine + alcohol activation clearly revealed that the rats were behaviourally intoxicated (see Fig. 2). RF, LF = right and left frontal leads; RP, LP = right and left parietal; RO, LO = right and left occipital. Calibrations: 100 μV , 1 s.

Signs of EEG-behavioural dissociation were observed transiently when ethanol alone was given. In all but one rat, both 1.5 g kg^{-1} and 2 g kg^{-1} induced behavioural intoxication during EEG activation that began within 2 to 6 min after injection and lasted at least 1.5 to 10 min for 1.5 g kg^{-1} and 1.5 to 5 min for 2 g kg^{-1} . There are a few reports of ethanol-induced excitatory effects in terms of EEG^{14–17} and in terms of enhanced evoked responses^{18–21}. Behavioural intoxication during such periods has not been reported, but my rats showed typical subjective signs of intoxication (mild ataxia, hypokinesia, bumping into objects, falling off the edge of a table, flaccid muscle tone and toppling over). During the EEG activation two rats were disconnected from the EEG machine and immediately given the catalepsy tests, which verified their behavioural intoxication.

The EEG-behavioural dissociation was still more conspicuous and readily demonstrated in rats given both physostigmine and ethanol. Physostigmine alone produced persistent EEG activation, beginning within 3 to 8 min and lasting at least 15 min (Fig. 1). When given 5 min before 2 g kg^{-1} ethanol, the EEG activation persisted in every rat for the full 15 min recording period, whereas this dose of ethanol always produced sustained EEG deactivation within

3 to 7 min (Fig. 1). Nonetheless, physostigmine-pretreated rats were clearly intoxicated. Physostigmine not only prevented the EEG deactivating property of ethanol, but to a lesser extent reversed it. Giving physostigmine 5 min after 2 g kg^{-1} of ethanol, at which time the EEG was usually deactivated, caused within several minutes EEG activations which alternated with deactivated periods in some rats. Again, rats displayed subjective signs of intoxication while their EEG was activated.

While it is likely that lower doses of physostigmine would have been less effective in blocking the EEG effect of ethanol the fact that a large dose did block shows there was an antagonism which was restricted to the EEG. A higher dose of physostigmine might in theory have also antagonised the intoxicated behaviour, but such a dose is toxic; in fact, signs of toxicity (shivering, teeth chattering) were universally present at the 0.2 mg kg^{-1} dose. The important point is that there is a dose of physostigmine at which EEG and behavioural effects of ethanol are clearly dissociable.

Physostigmine effects on the EEG were shown to be central, because neostigmine (0.2 and 0.3 mg kg^{-1}), which has the same action but does not cross the blood-brain barrier, failed to produce persistent EEG activation when given alone and of course also failed to prevent ethanol-induced deactivation.

In the battery of catalepsy tests, untreated rats did not show signs of catalepsy, but rats given 2 mg kg^{-1} ethanol failed to move from either the low or high platform (Fig. 2). The unexpected negative results on the cork test seemed due to compulsive slithering movements; rats moved immediately off of the corks and then resumed their hypokinesia. The results of the net test, though quantitatively the same as with saline, were qualitatively quite different. Sixty per cent of saline-injected rats stayed on the net more than 5 s before climbing down; ethanol-injected rats had so little muscle tone that they could not hold on at all.

Eserine, at the dosage used, caused mild teeth chattering, shivering, and hypokinesia (accounting for positive platform tests). The positive results of the net test reflected the skeletal muscle spasms which promoted clutching of the wire by all feet, making removal of the feet difficult.

When eserine and ethanol were given in combination, irrespective of which was given first, the rats displayed all the superficial signs of intoxication, such as ataxia, hypokinesia, and diminished postural tone. These signs were coexistent with the overt signs of eserine effects of shivering and teeth chattering. The failure of eserine to antagonise

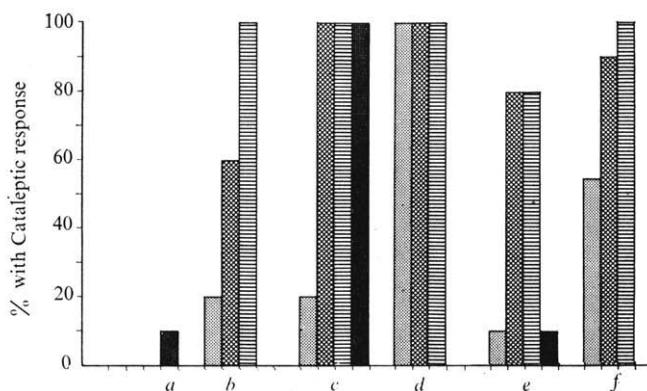


Fig. 2 Behavioural effects of *a*, saline; *b*, alcohol; *c*, eserine; *d*, alcohol and eserine; *e*, neostigmine; *f*, alcohol and neostigmine. Data from the 10 rats per group are expressed as the percentage which shows a cataleptic response, compared to the pretreatment performance of each rat. Vertical bars show the response after each treatment to (from left to right) cork test; 3 cm platform test; 9 cm platform test; net test.

onise the behavioural intoxication was clearly documented in the net test, where all rats fell off the net, because of hypotonia. A surprising and unexplained positive cork test was noted in all rats given the combined treatment.

Neostigmine alone caused rats to fail the net test; rats fell off from apparent weakness, suggesting that much of the body tension caused by eserine was not arising at the neuromuscular junction. As expected, neostigmine when combined with ethanol also caused failure on the net test. There may have been a significant failure ($P < 0.05$) on the cork test as well; neostigmine-ethanol treated rats seemed to have a compulsive squirming tendency but were too weak to move, whereas rats treated with eserine and ethanol made no attempts to move.

This work reveals that ethanol caused motor deficits more or less independently of cortical influences. Investigators may have been tempted to ignore subcortical and cerebellar sites as targets of ethanol action because ethanol has been reported to deactivate the EEG in animals with rostral brain stem transections²². A noncortical locus is also suggested from studies showing a similar dissociation of EEG and behavioural effects caused by stimulants such as amphetamine^{23,24}; such drugs prevented ethanol-induced deactivation of the EEG, but did not prevent behavioural signs of inebriation.

The present work also points to a noncholinergic basis of intoxication. A similar conclusion can be drawn from the recent report that pharmacological manipulation of the cholinergic system did not alter ethanol effects on a discriminated lever-press avoidance task²⁵.

Such demonstrations of a noncholinergic basis for behavioural intoxication are not really in conflict with a recent report that acetylcholine release is suppressed by ethanol²⁶. Such inhibition would account for ethanol-induced EEG deactivation, because acetylcholine mediates neocortical activation (reviewed in ref. 27). But the cortical action of ethanol could be secondary to general decreased neuronal activity, and in any case, it would not necessarily cause behavioural intoxication. Likewise, the motor deficits would not have to be mediated cholinergically. Some recent support for a noncholinergic basis for behavioural intoxication comes from the recent report that physostigmine and atropine did not affect ethanol withdrawal reactions in addicted mice, whereas such reactions were altered by pharmacological manipulation of brain catecholamines and γ -aminobutyric acid²⁸.

W. R. KLEMM

Department of Biology,
Texas A & M University,
College Station, Texas 77843

Received March 25; revised June 19, 1974.

- ¹ Bradley, P. B., and Hance, A. J., *Electroenceph. clin. Neurophysiol.*, **9**, 191-215 (1957).
- ² Burnam, W., and Erickson, C. K., *Fedn Proc. (Abstract)*, **28**, 262 (1969).
- ³ Erickson, C. K., and Burnam, W. L., *Agents Actions*, **2**, 8-13 (1971).
- ⁴ Pscheidt, G. R., Steiner, W. G., and Himwich, H. E., *J. Pharmac. exp. Ther.*, **144**, 37-44 (1963).
- ⁵ Gerebtzoff, M. A., *Archs int. Physiol.*, **51**, 365-378 (1951).
- ⁶ Klemm, W. R., *Electroenceph. clin. Neurophysiol.*, **21**, 365-372 (1966).
- ⁷ Loomis, A. L., Harvey, E. N., and Hobart, G., *Science*, **83**, 239-241 (1936).
- ⁸ Chertok, L., and Kramarz, P., *J. nerv. ment. Dis.*, **128**, 227-238 (1959).
- ⁹ Dement, W., *Electroenceph. clin. Neurophysiol.*, **10**, 291-296 (1958).
- ¹⁰ Klemm, W. R., *Am. J. vet. Res.*, **29**, 337-351 (1968).
- ¹¹ Klemm, W. R., and Hall, C. L., *J. Am. vet. med. Ass.*, **157**, 1640-1655 (1970).
- ¹² Klemm, W. R., and Hall, C. L., *Am. J. vet. Res.*, **33**, 2011-2025 (1972).
- ¹³ Otomo, E., *J. Neurol. Neurosurg. Psychiat.*, **29**, 383-390 (1966).

- ¹⁴ Hedenström, J., and Schmidt, O., *Dt. Z. Ges. gerichtl. Med.*, **40**, 234-251 (1951).
- ¹⁵ Horsey, W. J., and Akert, K., *Q. Jl Stud. Alcohol*, **14**, 363-377 (1953).
- ¹⁶ Hadji-Dimo, A. A., Ekberg, R., and Ingvar, D. H., *Q. Jl Stud. Alcohol*, **29**, 828-838 (1968).
- ¹⁷ Dolce, G., and Decker, H., *Res. Commun. chem. path. Pharmac.*, **3**, 523-534 (1972).
- ¹⁸ Masserman, J. H., *J. Pharmac. exp. Ther.*, **70**, 450-453 (1940).
- ¹⁹ Masserman, J. H., and Jacobson, L., *Archs Neurol. Psychiat.*, **43**, 334-340 (1940).
- ²⁰ Grenell, R. G., *Q. Jl Stud. Alcohol.*, **20**, 421-427 (1959).
- ²¹ Story, J. L., Eidelberg, E., and French, J. D., *Archs Neurol.*, **5**, 565-570 (1961).
- ²² Sauerland, E. K., and Harper, R. M., *Expl Neurol.*, **27**, 490-496 (1970).
- ²³ Greenberg, R. E., *Q. Jl Stud. Alcohol*, **28**, 1-10 (1967).
- ²⁴ Greenberg, R. S., and Goldstein, L., *Q. Jl Stud. Alcohol.*, **30**, 843-848 (1969).
- ²⁵ Graham, D. T., and Erickson, C. K., *Psychopharmacologia*, **34**, 173-180 (1974).
- ²⁶ Erickson, C. K., and Graham, D. T., *J. Pharmac. exp. Ther.*, **185**, 583-593 (1973).
- ²⁷ Jasper, H. H., in *Brain and Conscious Experience* (edit. by Eccles, J. C.), 256-282 (Springer, Berlin, 1966).
- ²⁸ Goldstein, D. B., *J. Pharmac. exp. Ther.*, **186**, 1-9 (1973).

Ultrastructural evidence for foetal liver injury induced by *in utero* exposure to small doses of methylmercury

THE ability of organomercury compounds, particularly methylmercury, to concentrate in tissues of fish and other animals at levels which are toxic for human consumption, is of increasing environmental concern¹⁻³. The propensity for mercury compounds to induce morphological, biochemical and enzymatic alterations in the kidneys and the central nervous system is well known⁴⁻⁸. Because the liver has been shown to accumulate a large fraction of mercury after administration of methylmercury, it should also be considered a target organ of methylmercury toxicity⁹. Hepatic injury and changes in several hepatic enzyme systems have been reported¹⁰.

Several investigators have shown that methylmercury compounds, when administered to pregnant mice, rats and monkeys rapidly pass through the placenta in the developing foetal tissues^{11,12}. Recently, ultrastructural evidence was reported for foetal nephropathy after *in utero* exposure to methylmercury (our unpublished results). We report here, however, the first ultrastructural demonstration of foetal liver injury induced by maternal exposure early in gestation to small doses of methylmercury.

Twenty-four pregnant female Sprague-Dawley rats (average body weight 250 g) were divided into four groups of six animals each, individually housed and permitted free access to water and Purina lab chow. Methylmercuric chloride (CH_3HgCl) was injected subcutaneously into groups 1, 2 and 3 in respective doses of 0.5, 0.75 and 1.0 mg on the ninth day of their gestational cycle. Group 4 control animals similarly received an equal volume of sterile physiological saline.

Neurological symptoms were not observed in pregnant female rats treated with methylmercury and no gross teratological malformations were noted in any of their offspring. Newborn pups were anaesthetised immediately with ether and their livers were excised surgically. Slices of liver were diced into small cubes and immersion fixed in mixture of 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3, for 4 h. They were then rinsed in phosphate buffer, post-fixed in buffered 2% osmium tetroxide, dehydrated in graded ethanol and propylene oxide and embedded in Epon. Thin sections were

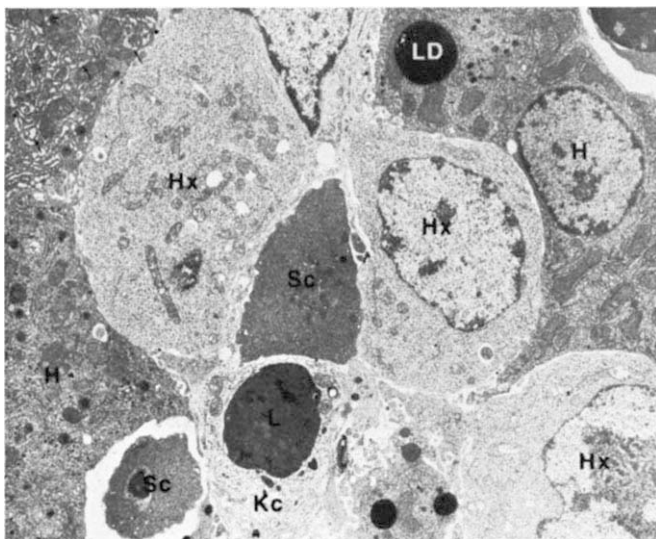


Fig. 1 Foetal hepatic parenchyma after maternal exposure to 1.0 mg CH_3HgCl (3.0 p.p.m. mercury) on the ninth day of gestation. Several hepatocytes (Hx), which are being extruded into the sinusoidal spaces, show an absence of general cellular organelles except for degenerating mitochondria and free ribosomes. Prominent dilation of endoplasmic reticulum (arrows) as well as sinusoidal casts (Sc) of cellular material were also observed. Kupfer cells (Kc) contain giant lysosomes (L). ($\times 1,860$).

counter-stained with uranyl acetate and lead citrate for electron microscopy. Thin coronal sections of the liver were fixed in buffered 10% formalin, dehydrated in ethanol and embedded in paraffin. Sections (5 μm) were stained with haematoxylin and eosin, and by the periodic acid-Schiff technique for light microscopic examination.

Light and electron microscopic evaluation of the foetal liver of animals treated *in utero* with sterile physiological saline revealed no significant morphological alterations. In contrast, foetuses from mothers exposed to methylmercury during gestation presented a consistent pattern of liver injury, the severity and extent of which were dose dependent. The most severe pathological alterations were observed in foetal livers exposed *in utero* to 1.0 mg of CH_3HgCl . At the

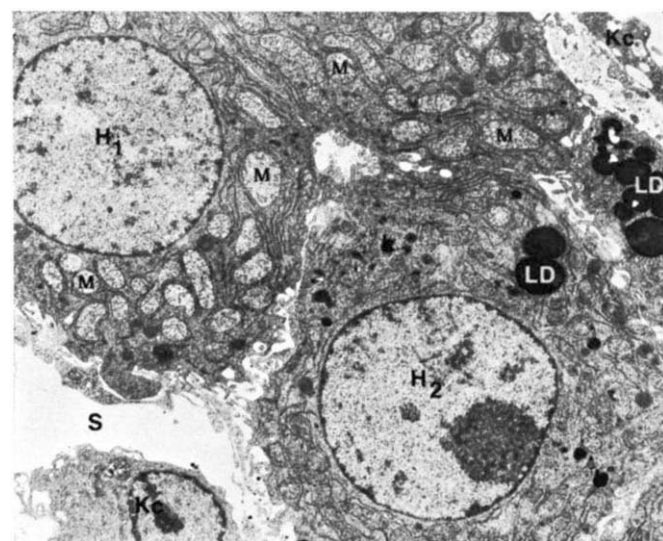


Fig. 2 Foetal hepatocytes after *in utero* exposure to 1.0 mg CH_3HgCl . An increase in lysosomes (L) and lipid droplets (LD) was observed in many hepatocytes. Extrusion of cytoplasmic material from hepatocytes into the sinusoidal spaces (S) was also observed (arrows). Swelling and floccular changes in mitochondria (M) in many hepatocytes (H_1) were observed while mitochondria in some hepatocytes (H_2) appeared normal. ($\times 4,650$).

light microscopic level these changes were confined primarily to hepatocytes and consisted of vacuolation, an increase in lysosomal profiles and in many cases, cellular necrosis.

Electron microscopic examination of foetal hepatocytes revealed, in addition to those changes observed by light microscope, mitochondrial degeneration, occasional accumulations of lipid droplets and dilatation of endoplasmic reticulum. Extrusion of hepatocyte cytoplasmic material into the sinusoidal spaces was also common (Fig. 1). Mitochondrial changes in hepatocytes consisted of an increasing electronlucid floccular matrix, diminution of cristae and occasional swelling (Fig. 2). Similar degeneration of hepatocytes, particularly hepatocyte mitochondria, has been observed in adult rats after methylmercury intoxication^{13,14}.

Several studies have shown that epithelial cells of renal proximal convoluted tubules accumulate administered mercury in lysosomes and mitochondria^{15,16}. Similar accumulation of mercury may be responsible for the hepatic lesions observed under our experimental conditions.

Our results demonstrate a significant causal relationship between maternal exposure to non-teratogenic doses of methyl mercury (1.5 to 3.0 p.p.m. mercury) during gestation and the resulting foetal liver injury. It certainly seems that early gestational exposure of pregnant female rats to minute amounts of methylmercury, which in themselves do not produce maternal neurological symptoms or gross teratology, may constitute considerable risk to the foetus.

RICHARD A. WARE

LOUIS W. CHANG

PETER M. BURKHOLDER

Department of Pathology,
University of Wisconsin Medical School,
Madison, Wisconsin 53706

Received May 20, 1974.

- 1 Ackefors, H., Lofroth, G., and Rosen, C. G., *Oceanogr. Marine Biol. Ann. Rev.*, **8**, 203-224 (1970).
- 2 Eyl, T. B., Wilcox, K. R., and Reizen, M. S., *Mich. Med.*, **69**, 880 (1970).
- 3 Hammond, A. L., *Science*, **26**, 788-789 (1971).
- 4 Gritzka, T. L., and Trump, B. F., *Am. J. Path.*, **52**, 1225-1278 (1968).
- 5 Ware, R. A., Burkholder, P. M., and Chang, L. W., *Am. J. Path.*, **21a** (1974).
- 6 Yoshino, Y., Mozai, T., and Nakao, K., *J. Neurochem.*, **13**, 1223-1230 (1966).
- 7 Chang, L. W., Ware, R. A., and Desnoyers, P. A., *Food Cosmet. Toxicol.*, **11**, 283-286 (1973).
- 8 Takeuchi, T., Matsumoto, H., Sasaki, M., Kambara, T., Shiraishi, Y., Hirata, Y., Nobuhiro, M., and Ito, H., *Kumamoto Med. J.*, **34**, 521-530 (1968).
- 9 Ware, R. A., Burkholder, P. M., and Chang, L. W., *Fedn Proc.*, **33**, 227 (1974).
- 10 Desnoyers, P. A., and Chang, L. W., *Fedn Proc.*, **32**, 838 (1973).
- 11 Khera, K. S., and Tabacova, S. A., *Food Cosmet. Toxicol.*, **11**, 245-254 (1973).
- 12 Takahashi, T., Kimura, T., Sato, Y., Shiraki, H., and Ukita, J., *J. Hyg. Chem.*, **17**, 93-107 (1971).
- 13 Desnoyers, P. A., and Chang, L. W., *Fedn Proc.*, **32**, 838 (1973).
- 14 Chang, L. W., and Yamaguchi, S., *Environ. Res.* (in the press).
- 15 Ware, R. A., Chang, L. W., and Burkholder, P. M., *Fedn Proc.*, **32**, 823 (1973).
- 16 Fowler, B. A., *Fedn Proc.*, **33**, 257 (1974).

Glutathione-oxytocin transhydrogenase of human placental origin with specificity towards neurohypophyseal hormones

THE rapid disappearance of endogenous neurohypophyseal hormones from the peripheral circulatory system has led many workers to study the mode of inactivation and site of degradation of these polypeptide hormones. Extracts of kidney¹, liver^{2,3},

pancreas⁴, uterus⁵, myometrium⁶, ovaries⁷ and brain and hypothalamic tissue⁸ have all been investigated for their ability to destroy the biological activity of oxytocin and vasopressin.

Of the enzyme systems capable of destroying oxytocin, those associated with pregnancy have been the most extensively studied⁹. Fekete¹⁰ first demonstrated that pregnancy serum inactivated the oxytocic activity found in the posterior pituitary lobe and it was later shown¹¹ that the inactivation of oxytocin and vasopressin extended to fresh extracts of human placentas. Although the placenta is rich in nonspecific aminopeptidases, the enzyme generally known by the name of oxytocinase is considered to be primarily responsible for the inactivation of oxytocin. This enzyme is a cystine aminopeptidase and causes hydrolytic cleavage of the cystine-tyrosine bond in positions 1 and 2, respectively, of the peptide hormone¹².

Branda and Ferrier¹³ have described the biological inactivation of oxytocin and deamino-oxytocin by placental extracts. These workers attributed the inactivation of deamino-oxytocin, which is not attacked by cystine aminopeptidase, to the presence of a thiol-protein-disulphide oxidoreductase followed by the action of tissue peptidases. We have examined placental extracts for a transhydrogenase enzyme system and determined its specificity towards the neurohypophysial hormones.

Placentas obtained from normal vaginal deliveries were cut into small pieces and after washing three times in 0.9% (w/v) saline at 4° C, were homogenised in an equal volume of distilled water using an Ultra Turrax T45 homogeniser (Janke and Kunkel Co., Germany). The particulate matter was removed by centrifugation at 1,400g for 10 min and further centrifugation of the supernatant at 105,000g for 10 min gave a clear placental extract which was used for enzyme assay using various compounds as substrates. Glutathione-protein-disulphide oxidoreductase activity was measured using the substrate at a concentration of 0.13 mM in Tris-HCl (0.1 M, pH 8.0) containing EDTA (1 mM). The assay method was based upon that described by Katzen and Stetten² in which NADPH (0.5 mg) is oxidised in the presence of reduced glutathione (16 mM) and glutathione reductase (5 IU). Enzyme activity is calculated from the change in absorbance min⁻¹ at 340 nm over a period of 5–6 min.

The glutathione-protein transhydrogenase activity against different disulphide-containing hormones and compounds is shown in Table 1. Of those proteins used as substrates, oxytocin and [8-arginine]-vasopressin were the most readily attacked whereas insulin was reduced to an extent of only 14% that of oxytocin. Cystine, homocystine and penicillamine disulphide were also readily reduced. As a result of the cleavage of the disulphide bond of oxytocin, we have named this enzyme system placental glutathione-oxytocin transhydrogenase.

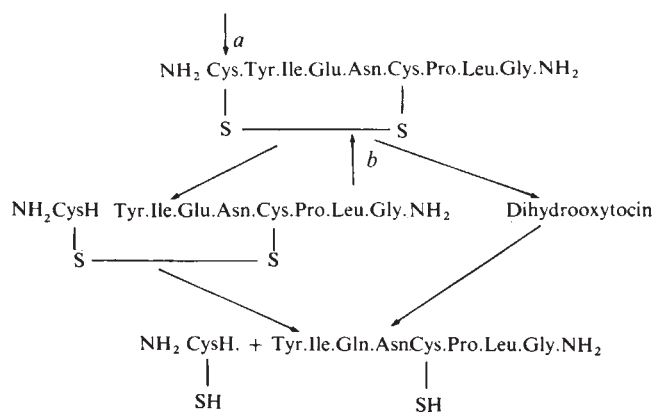


Fig. 1 Diagrammatic representation of the possible pathways involved in the enzymatic degradation of oxytocin by human placental tissue. *a*, Attack by cystine aminopeptidase; *b*, attack by placental glutathione-oxytocin transhydrogenase.

Table 1 Activity of placental glutathione-protein transhydrogenase with various protein hormones and compounds containing disulphide bonds

Substrate	Relative activity
Oxytocin	100
(8-arginine)-vasopressin	80
Bovine albumin	56
Human chorionic gonadotrophin	53
Human placental lactogen	48
Insulin	14
Cystine	190
Homocystine	140
N-benzoyloxycarbonyl cystine	40
Cystamine 2,2'-dithioethylamine	9
Cystine dimethyl ester	152
Cystine dibenzyl ester	30
Cystine di- <i>p</i> -nitroanilide	66
Cystine di- β -naphthylamide	49
Penicillamine disulphide	167
Dithiodioacetic acid	38
Dithiodipropionic acid	27
Dithiodibutyric acid	32
Dithiodibenzoic acid	38
Dithiodiacetic dibutyl ester	66

The activity is expressed as a percentage of the activity with respect to oxytocin.

Inactivation of vasopressin by the kidney¹⁴ and oxytocin by liver homogenates and tissue extracts⁴ is now considered to proceed through a transhydrogenase enzyme system³. The enzyme system present in the mammalian liver has been shown to possess a reactivity towards insulin² which is 14 times greater than the activity using oxytocin and vasopressin as substrates. In this context it is interesting to note that the liver also elaborates a protease which reacts specifically with insulin¹⁵.

Our findings show that the human placenta is similar to that of the liver in having both a proteolytic and transhydrogenase enzyme system specific towards the same substrate, that is cystine aminopeptidase and placental glutathione-oxytocin transhydrogenase. With the evidence of a transhydrogenase enzyme system being present in the placenta, the role of cystine aminopeptidase as being of prime importance in the degradation of oxytocin is now questionable. Oxytocin may be cleaved by two pathways as illustrated diagrammatically in Fig. 1. Cleavage of the disulphide bond by the transhydrogenase system can occur before peptidase action at the cystine-tyrosine residues or proteolytic action may be the initial step. It has not yet been established which of these sequence of events predominates.

This work was financed by the Medical Research Council of New Zealand.

C. W. SMALL
W. B. WATKINS

Postgraduate School of Obstetrics and Gynaecology,
University of Auckland,
Auckland, New Zealand

Received May 6; revised June 18, 1974.

- Walter, R., and Bowman, R., *Endocrinology*, **92**, 189–193 (1973).
- Katzen, H. M., and Stetten, D., *Diabetes*, **11**, 271–280 (1962).
- Rychlik, I., in *Oxytocin, Vasopressin and their Structural Analogues* (edit. by Rudinger, J.), 153–162 (Pergamon Press, Oxford, 1964).
- Beránková, Z., Rychlik, I., and Šorm, F., *Coll. Czech. chem. Commun.*, **25**, 2575–2580 (1960).
- Audrain, L., and Clauser, H., *Biochim. biophys. Acta.*, **38**, 494–501 (1960).
- Melander, S., *Acta obstet. gynaec. scand.*, **51**, 81–87 (1972).
- Fried, R., and Wüst, L., *Naturwissenschaften*, **41**, 238 (1954).
- Marks, N., Abrash, L., and Walter, R., *Proc. Soc. exp. Biol. Med.*, **142**, 455–460 (1973).
- Tuppy, H., in *Handbook of Experimental Pharmacology*, **XXIII** (edit. by Berde, B.), 67–129 (Springer-Verlag, Berlin, Heidelberg, New York, 1968).
- Fekete, K., *Endokrinologie*, **7**, 364–369 (1930).

- ¹¹ Hawker, R. W., *Q. Jl exp. Physiol.*, **41**, 301–308 (1956).
¹² Sjöholm, I., and Yman, L., *Acta Pharm. Suecica.*, **4**, 65–76 (1967).
¹³ Branda, L. A., and Ferrier, B. M., *Am. J. Obstet. Gynec.*, **109**, 943–947 (1971).
¹⁴ Dicker, S. E., and Greenbaum, A. L., *J. Physiol., Lond.*, **141**, 107–116 (1958).
¹⁵ Burghen, G. A., Kitabchi, A. E., and Brush, J. S., *Endocrinology*, **91**, 633–642 (1972).

Structural and crystallographic observations on hagfish insulin

INSULIN is a well defined globular protein made up of two small peptide chains linked together through disulphide bonds^{1,2}. It contains an unusually high proportion of hydrophobic residues and thus tends to readily self-associate^{3,4}. In many species the hormone forms dimers in solution and, in the presence of Zn²⁺ ions, hexamers^{4,5}. Recently, Hodgkin and coworkers^{6,7} have determined the crystal structure of the rhombohedral form of porcine Zn-insulin at 1.9 Å resolution^{6,7}. Two molecules of insulin occur in the asymmetric unit, related through an approximate twofold axis to form a nearly symmetrical dimer. Three such dimers in turn associate through coordination of histidine residues B10 with two zinc ions to form a nearly spherical hexamer. In spite of the availability of the amino acid sequences of a wide variety of vertebrate insulins^{2,8}, however, comparative studies on the detailed structure and modes of association of vertebrate insulins have been lacking.

We have recently studied the biosynthesis and structure of insulin in a cyclostome, the Atlantic hagfish (*Myxine glutinosa*)^{9,10}. The cyclostomes represent highly specialised survivors of the earliest vertebrates, the ostracoderms, and thus probably diverged from the gnathostomian vertebrates about 500 Myr ago. The islet organ in this species has been studied both structurally and functionally^{11,12}. Our interest in the associative behaviour of hagfish insulin arose particularly from the early finding, in primary structure analyses, that aspartic acid replaces histidine at position B10 (ref. 10). The crystallographic and sequence data presented here suggest that hagfish insulin forms dimers similar in structure to those of other insulins but does not form hexamers.

Islet organs were obtained from 4,200 hagfish at the Kristineberg Marine Biological Station (Fiskebäckskil, Sweden). Frozen islets were extracted twice with acid-ethanol, and the extract was fractionally precipitated by a modification of the method of Davoren¹³. The insulin-containing precipitate was gel filtered over a 115 × 5 cm BioGel P-30 column (BioRad Corporation)

in 3 M acetic acid. The insulin and proinsulin¹⁴ fractions were identified by means of a double-antibody radioimmunoassay, using a guinea pig anti-hagfish insulin antibody and iodinated hagfish insulin as a tracer. The purity of the BioGel fractions was assessed by polyacrylamide gel electrophoresis and by paper and cellulose thin-layer electrophoresis; the insulin (yield: about 10 mg) appeared to be at least 90% pure, the major contaminant probably being a desamido component.

Unlike most other insulins, hagfish insulin crystallised readily from a dilute acetone-containing citrate buffer near pH 6.0 in the absence of zinc. Insulin has previously been observed to crystallise in the absence of zinc only in acidic conditions (pH 2–4) (ref. 15). The crystallisation procedure was a modification of a micromethod used in the Novo Research (Bagsraerd, Denmark). Two solutions were employed: (I) 30 ml 1 M HCl, 160 ml acetone and water to 1 l; (II) 0.1 M sodium citrate containing 160 ml acetone l⁻¹. When solutions I and II were mixed in the proportions of 5 : 4, the final pH was about 6.0. 100 µg of hagfish insulin was placed in a small, conical Pyrex tube, was dissolved in 20 µl of solution I, and was heated briefly in a water bath (50°–55° C). 16 µl of solution II was then added, and the solution was mixed and warmed to 50°–55° C and then was allowed to cool to 25° C over a period of 12 h. In these conditions large tetragonal crystals formed at 25° C or at 4° C within 2 d, even in the presence of 0.01 M EDTA. Compositional and electrophoretic analysis of washed crystals revealed only hagfish insulin and the putative desamido form.

The crystals were clear bipyramids up to 0.5 mm in length. X-ray diffraction data on wet mounted crystals showed that these were tetragonal, space group P4₁2₁2 (or the enantiomorph) (ref. 16), with cell dimensions of $a = 38.51$ Å, $c = 85.37$ Å. If the molecular weight of the monomeric protein is taken to be 5,967, then eight molecules of protein in the cell would give as a specific volume $V = 2.65$ Å³ per dalton. This value is within the range for proteins reported by Matthews¹⁷, but is considerably larger than the 1.9 Å³ per dalton calculated for rhombohedral Zn-insulin, suggesting looser packing. With eight insulin molecules per tetragonal unit cell in P4₁2₁2 or P4₃2₁2, there is one insulin molecule in the asymmetric unit which may form a perfect dimer across the crystallographic twofold axis, but which could not form a hexamer of the type found by Hodgkin *et al.* in porcine zinc-insulin crystals. We have collected diffraction data to 2.5 Å on these crystals, and are collaborating with Professor Hodgkin's group in Oxford on a complete crystal structure analysis.

The methods used in our laboratory for amino acid sequence analysis have been reported elsewhere¹⁸. Compositional data for hagfish insulin have been reported previously¹⁹.

A Chains		1	5	10	15	20	21
Hagfish		Gly-Ile-	Val-Glu-Gln-Cys-Cys-His-Lys-Arg-Cys-Ser-Ile-	Tyr-Asn-Leu-Gln-Asn-Tyr-Cys-Asn			
Porcine		Gly-Ile-	Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-	Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn			
Other residues			His Asp	Ala Gly Pro Arg Val Asn Thr	Asp Arg Phe Asp Asn Lys His Thr		
B Chains		0	1	5	10	15	20
Hagfish			Arg-Thr-Thr-Gly-His-Leu-Cys-Gly-Lys-Asp-Leu-Val-Asn-Ala-Leu-Tyr-Ile-	Ala-Cys-Gly-			
Porcine			Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-				
Other residues		Val Met	Ala Pro Pro Arg	Pro Asn	Asp Thr	Ser	Gln
			Ala Lys Pro Ser Ala				
		21	25	30	31		
Hagfish		-Val-Arg-Gly-Phe-Phe-Tyr-Asp-Pro-Thr-Lys-Met					
Porcine		-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala					
Other residues		Asp Asp	Ser Ser Met Ser (Arg)	Ile Thr	Gln Asp		

Fig. 1 The amino acid sequence of hagfish and porcine insulins, with substitutions at each position from other known insulins shown for comparison. The additional residue of the hagfish B chain might be the result of either an insertion or deletion event at position 29 or 30 or a single base change within the codon corresponding to the first residue (Arg) of the interchain connecting segment of the porcine proinsulin.

Amino acid sequence studies were carried out on the S-carboxymethylated or oxidised chains of hagfish insulin separated by electrophoresis or gel filtration. These will be reported in detail elsewhere (J. D. P., S. O. E., and D. F. S., unpublished). The results of these studies provide clues to some aspects of the three-dimensional structure of hagfish insulin. A comparison of the sequence of hagfish insulin with that of porcine insulin and the cumulative data on individual amino acid substitutions in all known vertebrate insulins⁸ is shown in Fig. 1.

The most striking feature of the hagfish insulin sequence is that, except at position B18, every residue which is invariant among the other known insulins is also conserved in the hagfish sequence. These invariant residues include all of the cysteines and most of the hydrophobic residues present at the dimer interface in porcine insulin crystals (that is, B11, B12, B16, B24-27). The substitution of aspartic acid for histidine at position B10 is an important difference that may account for the absence of hexamers in the hagfish insulin crystals. (Substitutions at this position are also known to occur in the insulins of the guinea pig and coypu⁸, but crystallisation studies have not been reported. Islet tissues from these do not contain much zinc²⁰.) The substitution of phenylalanine and threonine for the hydrophobic residues usually present at positions B1 and B2 may also interfere with hexamer formation. Residues A8 to A10 make up a very basic centre similar to that found in some fish insulins². Sequence homologies (Fig. 1) suggest that the helical regions of the A chain (A2 to A8 and A13 to A20) are similar in the two species and that the α -helical segment of the B chain (B9 to B19) may also be present in the hagfish.

From this analysis there emerges a predicted three-dimensional structure of hagfish insulin which is similar to that of known mammalian insulins. Major sequence changes are localised in the extended chain regions, positions A8-A12 and the amino end of the B chain (B1-B10). The stretches of α -helix, the cross bridges, and the residues involved in dimerisation, all of which are important structural determinants in porcine insulin, appear to be conserved in hagfish insulin. On the other hand, the zinc binding site and other features that may play a role in stabilising a hexamer structure are not well conserved in hagfish insulin, which is in keeping with the tetragonal symmetry of these crystals. These predictions appear to be borne out also by a recent study at 6 Å resolution, comparing the structures of hagfish and porcine insulins²¹. The functional significance of these differences in terms of either efficient insulin formation or tissue binding are still not clear. The conservation of features related to dimerisation, however, may mean that the dimer is a significant functional entity. (Although guinea pig insulin has been reported not to form dimers in solution²², the necessary regions for dimerisation are present and self-association may occur, for example, at an appropriate tissue-receptor site.) Further crystallographic analysis of hagfish insulin combined with studies of its biological activity¹⁹ should help to more clearly define those regions and structural features of the insulin molecule that are necessary for its effective receptor interaction and biological activity²¹.

This work was supported by the United States Public Health Service (USPHS) and grants from the Swedish Medical Research Council. J. D. P. is a predoctoral trainee of the USPHS. We thank Professor B. Swedmark and the staff at the Kristineberg Marine Biological Station for material and working facilities, Dr A. Tulinsky (Michigan State University) for assistance in collecting the X-ray data and Miss Roberta Erfurth and Mrs Adelaide Jaffe for assistance in preparing this manuscript.

J. D. PETERSON
C. L. COULTER
D. F. STEINER

Departments of Biochemistry and Anatomy,
The University of Chicago,
Chicago, Illinois 60637

S. O. EMDIN
S. FALKMER

Department of Pathology,
University of Umea School of Medicine,
Umea, Sweden

Received May 20; revised July 1, 1974.

- ¹ Ryle, A. P., Sanger, F., Smith, L. F., and Kitai, R., *Biochem. J.*, **60**, 541-556 (1955).
- ² Dayhoff, M. O., Atlas of Protein Sequence and Structure, **5** (Biomedical Research Foundation, Bethesda, 1972).
- ³ Fisher, H. F., *Proc. natn. Acad. Sci., U.S.A.*, **51**, 1285-1291 (1964).
- ⁴ Humbel, R. E., Bosshard, H. R., and Zahn, H., in *Handbook of Physiology-Endocrinology I* (edit. by Steiner, D. F., and Freinkel, N.), 111-132 (Williams and Wilkins, Baltimore, 1972).
- ⁵ Frank, B. H., and Veros, A. J., *Biochem. biophys. Res. Commun.*, **38**, 284-289 (1970).
- ⁶ Blundell, T., Dodson, G., Hodgkin, D., and Mercola, D., *Adv. Protein Chem.*, **26**, 279-402 (1972).
- ⁷ Blundell, T. L., Dodson, G. C., Dodson, E., Hodgkin, D. C., and Vijayan, M., *Rec. Prog. Horm. Res.*, **27**, 1-40 (1971).
- ⁸ Smith, L. F., *Am. J. Med.*, **40**, 662-666 (1966).
- ⁹ Peterson, J. D., Steiner, D. F., Emdin, S. O., Ostberg, Y., and Falkmer, S., *Fedn Proc.*, **32**, 577 (1973).
- ¹⁰ Steiner, D. F., Peterson, J. D., Tager, H. S., Emdin, S. O., and Falkmer, S., *Am. Zool.*, **13**, 591-604 (1973).
- ¹¹ Falkmer, S., Thomas, N. W., and Boquist, L., in *Chemical Zoology* (edit. by Florkin, M., and Scheer, B.), **8**, 195-257 (Academic, New York, 1974).
- ¹² Falkmer, S., Emdin, S. O., Havu, N., Lundgren, G., Marques, M., Ostberg, Y., Steiner, D. F., and Thomas, N. W., *Am. Zool.*, **13**, 625-638 (1973).
- ¹³ Davoren, P. R., *Biochim. biophys. Acta*, **63**, 150-153 (1962).
- ¹⁴ Steiner, D. F., Kemmler, W., Clark, J. L., Oyer, P. E., and Rubenstein, A. H., in *Handbook of Physiology-Endocrinology I* (edit. by Steiner, D. F., and Freinkel, N.), 175-198 (Williams and Wilkins, Baltimore, 1972).
- ¹⁵ Low, B. W., and Shoemaker, C. B., *Acta Cryst.*, **12**, 893-896 (1959).
- ¹⁶ *International Tables for X-ray Crystallography* (edit. by Henry, N. F. M., and Lonsdale, K.), **1** (Kynoch Press, Birmingham, England, 1965).
- ¹⁷ Matthews, B. W., *J. molec. Biol.*, **33**, 491-497 (1968).
- ¹⁸ Peterson, J. D., Nehrlich, S., Oyer, P. E., and Steiner, D. F., *J. biol. Chem.*, **247**, 4866-4871 (1972).
- ¹⁹ Weitzel, V., Marques, M., Ostberg, Y., Hahn, J., and Martini, O., *Hoppe-Seyler's Z. Physiol. Chem.*, **348**, 525-532 (1967).
- ²⁰ Falkmer, S., in *Proc. seventh Cong. Int. Diabetes Fed.* (edit. by Rodriguez, R. R., and Vallance-owen, J. J.), 219-225 (Excerpta Medica, Amsterdam, 1971).
- ²¹ Cutfield, J. F., Cutfield, S. M., Dodson, E. J., Dodson, G. G., Hodgkin, D. C., and Sabesan, M. N., *J. molec. Biol.* (in the press).
- ²² Zimmerman, A. E., Kells, D. I. C., and Yip, C. C., *Biochem. biophys. Res. Commun.*, **46**, 2127-2133 (1972).

Changes in histone methylase activity of rat brain and liver with ageing

No function has been found for the methylation of the ϵ -N-amino group of lysine in certain histones that have been demonstrated in thymus gland, liver and erythrocytes as well as several other organs¹. It has been suggested, however, that this modification of the histone polypeptide effects the DNA-histone interaction and may be involved in regulating gene expression¹. Methylation appears to be very specific, occurring after histone synthesis is complete². Methylation of certain histones can be correlated with development. Gershey *et al.* reported that 3-methylhistidine was formed more rapidly in immature erythroid cells than in mature erythrocytes³, and Borun *et al.* found that in developing HeLa cells the lysyl residues of F_{2b}, F₃ and F_{2a1} histones were methylated mainly in the G₂ phase and during mitosis⁴. Methylation of rat brain histones also seems to depend on age. After the intraperitoneal injection of L-¹⁴CH₃-methionine into different age classes of rats we found that only trace amounts of ¹⁴C-methyl were incorporated into brain histones after maturity⁵. If L-³H-lysine and L-¹⁴CH₃-methionine are administered to newborn rats biphasic type decay curves (³H and ¹⁴C) are obtained for all

Table 1 Age dependence of histone methylation in rat brain and liver nuclei

	Brain		Liver	
	d.p.m. mg ⁻¹	pmol mg ⁻¹	d.p.m. mg ⁻¹	pmol mg ⁻¹
Newborn	142,000	65	65,000	29
11	260,000	118	51,000	23
21	179,000	81	72,000	32
83	90,000	40	—	—
365	81,000	36	45,000	20
900	26,100	12	28,000	13

Nuclei from 15 g of brain or liver from different age groups were isolated and suspended in 0.32 M sucrose containing 0.1 M potassium phosphate buffer, pH 7.5 and 1.0 mM MgCl₂. The nuclei were incubated with 100 μ Ci C³H₃-S-adenosylmethionine (1.0 Ci mol⁻¹) for 30 min at 37° C. The reaction was terminated by chilling to 0° C and the histones purified as previously described⁶. The histones were precipitated with acetone and the protein concentration and radioactivity were determined.

histone subfractions. During brain development (up to 50 d) the arginine-rich histones have a half-life of 32 d while no turnover is observed after maturity as measured by either ³H or ¹⁴C decay. We now find that the level of the histone methylases and the ability of brain histones to accept methyl groups decreases during ageing. Freshly prepared brain and liver nuclei from rats of various ages were incubated with C³H₃-S-adenosylmethionine. The reaction mixtures were chilled to 0° C, the nuclei centrifuged down and extracted three times with 0.14 M NaCl. The residue was washed with methanol-chloroform, acetone, acetic acid (pH 3.0) and the histones extracted with 0.4 M HCl⁶. When L-¹⁴C-lysine was added to the reaction mixture no ¹⁴C was incorporated into the resultant histones excluding *in vitro* synthesis of histone in our system.

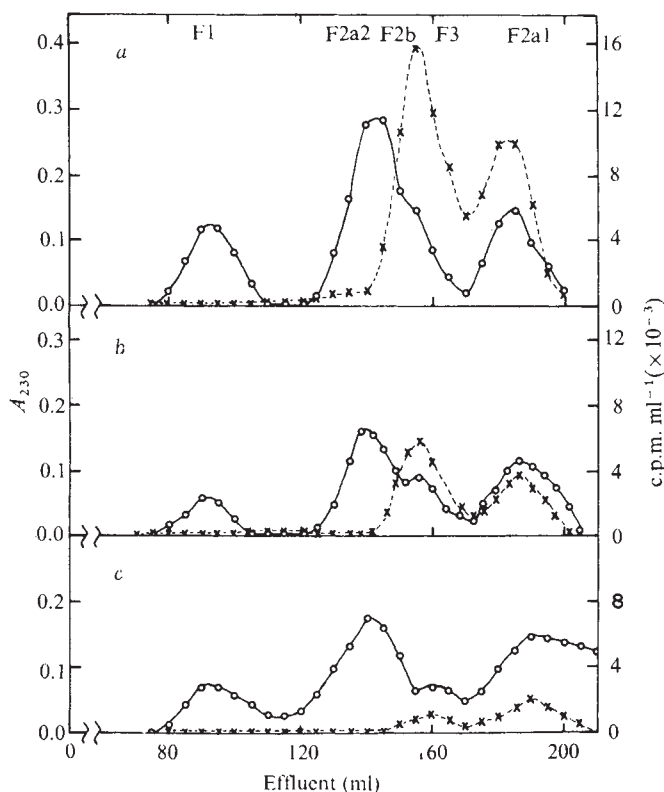


Fig. 1 Bio-Gel P-10 column chromatography of brain histones. From 6 to 8 mg of labelled histones prepared from brain nuclei as outlined under Table 1 were dissolved in 10 mM HCl containing 6 M urea. This solution was applied to a Bio-Gel P-10 column (1.6 × 350 cm) and the histones eluted with 10 mM HCl. Fractions of 3.5 ml were collected. Samples of 0.2 to 1.0 ml were taken from each tube for the determination of radioactivity. Histones were from newborn rats (a), 83-d-old rats (b) and 900-d-old rats (c).

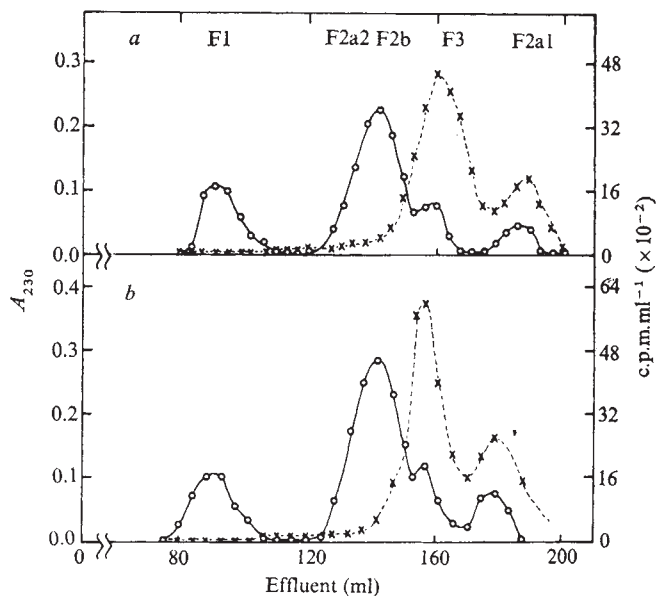


Fig. 2 Bio-Gel P-10 column chromatography of liver histones from newborn rats (a) and 900-d-old rats (b). The labelled histones prepared from liver nuclei (Table 1) were fractionated on Bio-Gel P-10 as outlined under Fig. 1.

There was an increase in methylation of the histones in brain nuclei during the first few days after birth (Table 1). After approximately 11 d the activity decreased progressively throughout the life of the animal. The extent of methylation of histones in intact brain nuclei was three to four times greater during the various developmental stages than in the liver nuclei, while the decrease in activity with ageing was more pronounced in brain nuclei.

After fractionation on Bio-Gel P-10 the F₃ and F_{2a1} histones were the only components from brain and liver nuclei found to be highly methylated (Figs 1 and 2). The amount of labelled methyl groups incorporated into the F₃ and F_{2a1} histones decreased during ageing in both liver and brain while the degree of methylation of F₃ was always greater than that of F_{2a1}.

After hydrolysis, the products of methylation in the F_{2a1} and F₃ histones were chiefly monomethyllysine and dimethyllysine (Table 2). No methylated arginine or histidine was found in any of the histones analysed. In all cases, except the F_{2a1} from liver nuclei of 2.5-yr-old rats, there was more monomethyllysine than dimethyllysine. By direct quantitative analysis we find that 8.1% of the lysyl residues in rat liver F_{2a1} histones are methylated, mainly as ϵ -N-dimethyllysine. In F₃ methyl groups are present on 13.4% of the lysyl residues mainly as mono and trimethyllysine in a ratio of 1:1. Tidwell *et al.* have also found that ϵ -N-dimethyllysine is the predominant form in F_{2a1} from rat liver². The disproportionately high content of monomethyllysine in F_{2a1} and F₃, as measured by pmol ³H incorporated (Table 2), would be expected if the methyl groups were added sequentially.

Table 2 Distribution of methylated lysyl residues in liver histones

Histones	pmol mg ⁻¹ histone		
	Monomethyllysine	Dimethyllysine	Trimethyllysine
F ₃ (newborn)	20.64	8.30	0.70
F ₃ (2.5-yr-old)	14.42	5.39	0.19
F _{2a1} (newborn)	16.23	2.48	0.05
F _{2a1} (2.5-yr-old)	5.59	11.70	0.00

The labelled liver histones fractionated on Bio-Gel P-10 (Fig. 1) were hydrolysed in 6.0 M HCl for 24 h at 110° C and chromatographed on a column of Beckman PA-35 resin as described previously³. The level of methylated lysine was calculated from the amount of ³H present under the peaks.

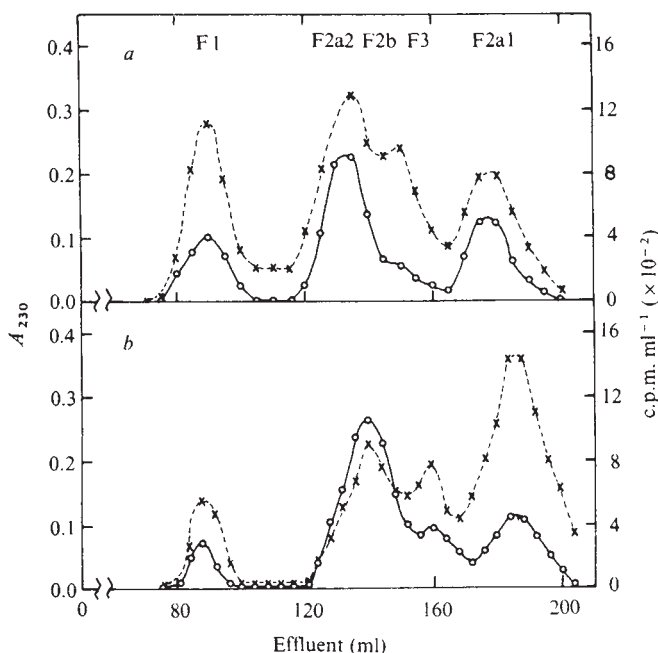


Fig. 3 Bio-Gel P-10 column chromatography of brain (a) and liver (b) histones after incubation with methylases from the same organ. Brain histones from 11-d-old rats and liver histones from 21-d-old rats were incubated with methylases from the same organ of 2.5-yr-old animals in the presence of C^3H_3 -S-adenosylmethionine as described under Tables 3 and 4. The histones were fractionated on Bio-Gel P-10 as described under Fig. 1.

The apparent decrease in methylation of brain and liver histones with age could be due to a decrease in the level of histone methylases and/or the availability of sites on the histones to accept methyl groups. To delineate between these two possibilities, histone methylases were assayed in young and old animals using as substrates histones prepared from the same organs.

As Tables 3 and 4 show, the histone methylase was considerably more active in extracts of liver and brain nuclei of young animals. There was no significant difference in the ability of histones prepared from liver nuclei of young or old animals to accept methyl groups. Histones prepared from the brains of young rats were somewhat better methyl group acceptors than those from old brains. Our data from both intact nuclei and soluble enzyme preparations agree with those of Paik and Kim⁸, who found that histone methylase III, prepared from rat brain, increased after birth until the 10th day and decreased significantly thereafter. They used calf thymus histone as

Table 3 Histone methylase activity in the brain of young and old rats

Methylase source	Histone source	Specific activity d.p.m. mg ⁻¹	S-adenosyl- methionine utilised pmol mg ⁻¹
1.0 mg	1.0 mg		
Old	Old	11,200	50
Old	Young	17,500	79
Young	Old	24,500	111
Young	Young	32,400	149

The enzyme fractions and histones were prepared from 11 and 900-d-old rats⁷. The reaction mixtures contained 50 μ Ci C^3H_3 -S-adenosylmethionine, 20 mM phosphate buffer, pH 7.5, 1.0 mM dithiothreitol and 1.0 mg each of histone and enzyme protein from either old or young animals. After incubation for 30 min at 37 °C the reaction was stopped by adjusting the pH to 9.7 with NaOH and the addition of urea 8 M⁹. The reaction mixtures were applied to DEAE-cellulose columns (1.0 $\times$ 20) previously equilibrated with 10 mM glycine NaOH buffer, pH 9.7, containing 8 M urea. The histones were eluted with the same buffer, dialysed against 10 mM HCl and lyophilised. The histones were heated at 70 °C for 20 min in 1.1 M trichloroacetic acid (TCA), collected on Millipore filters and washed with 1.1 M TCA. The precipitates were suspended in 10 ml Bray's counting fluid⁹ and the radioactivity was determined using a Packard liquid scintillation counter.

Table 4 Histone methylase in the livers of young and old rats

Methylase source	Histone source	Specific activity d.p.m. mg ⁻¹	S-adenosyl- methionine utilised pmol mg ⁻¹
1.0 mg	1.0 mg		
Old	Old	7,130	32
Old	Young	6,100	28
Young	Old	22,760	102
Young	Young	25,000	116

The enzyme fractions and histones were prepared from 21 and 900-d-old rat liver nuclei. All other conditions were the same as outlined under Table 3.

substrate and would not have observed differences in the acceptor ability of histones prepared from animals of different ages.

The labelled histones from our experiments were fractionated on Bio-Gel P-10 and the distribution of methylated components was determined. The profiles of brain and liver histones from young animals methylated by the enzyme from old animals are given in Fig. 3. All histone subfractions were highly methylated. When methylases from brain and liver nuclei of young animals were used the resultant specific activities of the histones were greater but the distribution of label in the subfractions did not change.

The distribution of labelled methyl groups in these histones differed significantly from what we observed in experiments with intact nuclei. Apparently when the histones are associated with the DNA few, if any, of the lysyl residues on the F₁, F_{2a2} or F_{2b} histones are accessible to the methylases. Apparently the specificity for the methylation of the lysyl residues in the polypeptide chain is lost when histones are dissociated from DNA. These experiments suggest that some of the specificity for methylation resides within the arrangement of the histones on DNA and that methylation occurs after the histones are bound to the DNA.

This work was supported by grants from the National Institutes of Health.

CATHERINE T. LEE
JOHN A. DUERRE

Department of Microbiology,
Ireland Research Laboratories,
University of North Dakota,
Grand Forks, North Dakota 58201

Received April 16; revised June 27, 1974.

- Allfrey, V. G., in *Histones and nucleohistones* (edit. by Phillips, D. M. P.), 241 (Plenum, London, New York, 1971).
- Tidwell, T., Allfrey, V. G., and Mirsky, A. E., *J. biol. Chem.*, **243**, 707 (1968).
- Gershey, E. L., Haslett, G. W., Vidali, G., and Allfrey, V. G., *J. biol. Chem.*, **244**, 4871 (1969).
- Borun, T. W., Pearson, D., and Paik, W. K., *J. biol. Chem.*, **247**, 4288 (1972).
- Duerre, J. A., and Lee, C. T., *J. Neurochem.*, **23**, 541 (1974).
- Duerre, J. A., and Gaitonde, M. T., *J. Neurochem.*, **18**, 1921 (1971).
- Burdon, R. H., and Garven, E. V., *Biochim. biophys. Acta*, **232**, 371 (1971).
- Paik, W. K., and Kim, S., *Biochim. biophys. Acta*, **313**, 181 (1973).
- Bray, G. A., *Analyt. Biochem.*, **1**, 279 (1960).

Immunological distinction between the possible origins of enzymatic activity in a polypeptide fragment of staphylococcal nuclease

WE have used an immunological approach to demonstrate that the residual enzymatic activity in a large polypeptide fragment of staphylococcal nuclease is an intrinsic property of the fragment and not an artefact due to contamination with intact enzyme. These results are consistent with present concepts of the folding of polypeptide fragments to form

structures similar to the native protein and may offer a method for the investigation of other macromolecular systems.

Staphylococcal nuclease consists of a single polypeptide chain of 149 amino acids¹ and has nucleolytic activity towards DNA which is readily measurable spectrophotometrically. After trifluoroacetylation to block free amino groups, nuclease can be cleaved by trypsin preferentially at arginine residue 126 (ref. 2). Removal of the blocking groups and subsequent chromatographic purifications yield

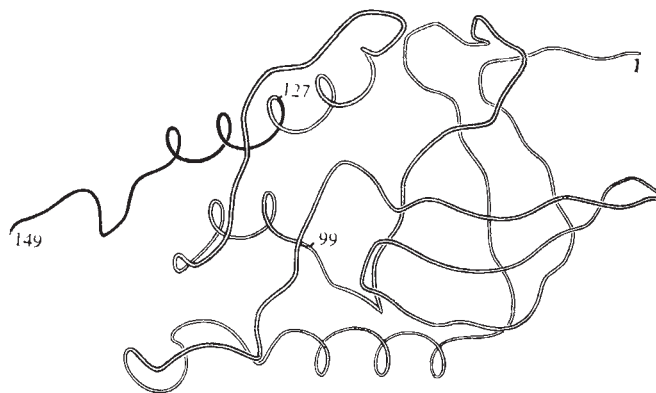


Fig. 2 An artist's representation of nuclease with the sequence between amino acids 127 and 149 shaded to indicate the molecular localisation of the antigenic determinant of anti-(127-149)_N antibodies.

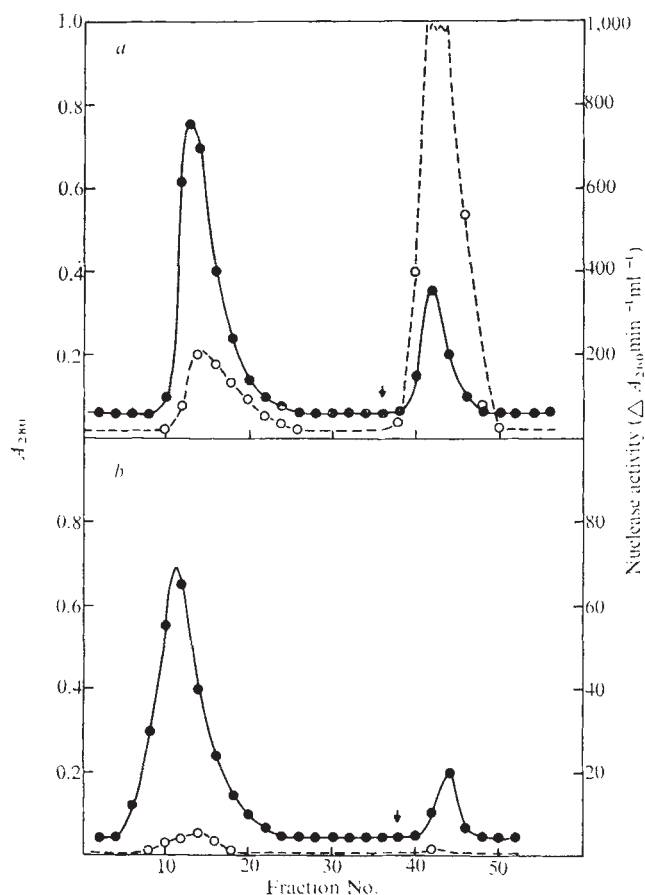


Fig. 1 Affinity chromatography of nuclease (a) and of (1-126) (b) on Sepharose columns bearing the substrate analogue thymidine diphosphate³. Point of buffer change to effect elution is indicated by arrows in both figures. ●—●, Optical density; ○—○, enzymatic activity. Note that the acid eluate in (a) has the full activity of pure nuclease (a small amount of active material is not bound in these conditions), while the 1-126 adsorbed to the column is of the same activity as that which passes through.

a polypeptide fragment consisting of amino acid residues 1 to 126 (previously designated (1-126) (ref. 3, 4)). This fragment contains all amino acid residues of known importance to the active site. Spectral measurements of (1-126) indicate an absence of the characteristic ordered secondary structure of nuclease, which suggests the importance of long range tertiary interactions of the distal fragment in the proper folding of the native nuclease molecule¹.

But, (1-126) preparations are not devoid of enzymatic activity: affinity chromatography on pdTp-Sepharose⁶ generally yields a relatively inactive run-through peak followed by pure, active nuclease in the acid-eluted peak (Fig. 1a). Chromatography of (1-126) preparations yielded peaks in which both the run-through and acid-eluted peaks showed a similar activity/protein ratio (Fig. 1b). Multiple passages of the first peak through the affinity column decreased the

activity of this material to a level about 0.11% that of nuclease, on a molar basis, and which could not be decreased by further passages. It is important to know whether this activity is due to contaminating native nuclease or is intrinsic to (1-126). Such intrinsic activity would imply that the correct folding of this fragment to generate an enzymatically active site must occur spontaneously, albeit to a small extent, even in the absence of the tertiary stabilising influence provided by residues 127-149. Unfortunately, most analytical methods do not distinguish between these explanations for the activity of (1-126). The amount of contaminating nuclease that would suffice to produce 0.11% enzymatic activity is too small to detect by analytical gels or by the unique spectral characteristics of nuclease. Conversely, the minute fraction of folded (1-126) that might be sufficient to generate this level of enzymatic activity would not be detected spectroscopically.

We have therefore used an immunological approach. We prepared and fractionated antibodies to nuclease as previously described⁶. Briefly, antibodies to the active enzyme were fractionated on Sepharose columns to which selected fragments of nuclease had been bound covalently. The antibody fraction resulting from absorption to and elution from Sepharose-(99-149) and then Sepharose-(127-149) has been called anti-(127-149)_N, the subscript N indicating that the antibodies were prepared by immunising with intact native nuclease. Such antibodies combine with nuclease to produce an enzymatically inactive antibody-antigen complex⁷. But, since the reaction presumably involves an antigenic determinant in the region 127-149 of the nuclease molecule (Fig. 2), such antibodies ought not to react with (1-126).

The effects of anti-(127-149)_N antibodies on the activities of nuclease and (1-126) are illustrated in Fig. 3. An amount of antibody (5 μg) sufficient to inactivate 12.5 ng of nuclease did not inhibit the activity of 5 μg of (1-126), which contained comparable enzymatic activity. As a control against the possibility that the larger amount of protein in the latter case might have interfered with the antibody-antigen interaction, the effect of anti-(127-149)_N on a mixture of nuclease and (1-126) was also studied (Fig. 3). The antibodies were found to reduce the activity of such mixtures to that which would be predicted for the amount of (1-126) present.

Our results indicate then, that the residual enzymatic activity associated with polypeptide fragment (1-126) is an intrinsic property of that fragment and not due to contamination with nuclease. We have shown previously that many features of the interactions of purified monospecific anti-nuclease antibodies with fragments of nuclease can be interpreted by a model in which the fragments are presumed to exist in solution in conformational equilibrium be-

tween various disordered or random conformations and the conformation assumed by the corresponding amino-acid sequence in the native protein⁸. Our present results can also be interpreted by such a model, since a consequence of the native folding of the polypeptide fragment (1-126) would be the native orientation of amino acids of the enzymatic active site, leading to a level of residual activity related to the folded fraction. The existence of fragments sufficiently folded to yield activity but with altered K_m and V_{max} values might of course affect the numerical relationship between activity and folding.

The hypothesis that the primary sequence of a protein determines its native three-dimensional structure has been substantiated for various proteins⁹. However, the mechanism by which the primary sequence is translated into correct folding of a protein is unclear. Preferred conformations

formational equilibria, since the detection of folded forms depends on the sensitivity of the method of study relative to the position of the equilibrium. In terms of mechanism, polypeptides, either on or off ribosomes, can be envisaged as continually trying out different possible three-dimensional conformations. The degree to which native format is achieved by an incomplete protein fragment might then be considered a reflection of the relative thermodynamic stability of this conformation as against that of other possible conformations of the same polypeptide chain. In the intact protein or in a fairly large polypeptide fragment of that protein, such as (1-126), the ability of the native conformation of the polypeptide sequence to interact with distant portions of the molecule might be expected to increase the thermodynamic stability of that particular conformation. Clearly there are many possible three-dimensional conformations of a polypeptide chain of 126 amino acids. Thus even the small percentage folding which we propose as the explanation for the intrinsic enzymatic activity of (1-126) represents a considerable restriction of possible conformations of this polypeptide. Presumably the sequence of (1-126) permits some, but not all, of the tertiary interactions which lend stability to the native conformation of the intact protein.

There are many examples, especially among polypeptide hormones (for example ref. 13), where biological activity may be retained in fragments produced by cleavage or synthesis. The use of antibodies with specificity for limited regions may help clarify the mechanisms of these phenomena.

We thank Dr Hiroshi Taniuchi for valuable discussions.

DAVID H. SACHS

ALAN N. SCHECHTER

ANN EASTLAKE

CHRISTIAN B. ANFENSEN

Immunology Branch,
National Cancer Institute and Laboratory of Chemical
Biology,
National Institute of Arthritis, Metabolism and
Digestive Diseases,
National Institutes of Health,
Bethesda, Maryland 20014

Received April 18, 1974.

- ¹ Anfinsen, C. B., Cuatrecasas, P., and Taniuchi, H., in *The Enzymes*, 4 (edit. by Boyer, P. D.), 177 (Academic Press, New York, 1971).
- ² Cuatrecasas, P., Fuchs, S., and Anfinsen, C. B., *J. biol. Chem.*, **242**, 1541 (1972).
- ³ Taniuchi, H., Anfinsen, C. B., and Sodja, A., *J. biol. Chem.*, **244**, 3864 (1969).
- ⁴ Taniuchi, H., and Anfinsen, C. B., *J. biol. Chem.*, **246**, 2291 (1971).
- ⁵ Cuatrecasas, P., Wilchek, M., and Anfinsen, C. B., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 636 (1968).
- ⁶ Sachs, D. H., Schechter, A. N., Eastlake, A., and Anfinsen, C. B., *J. Immun.*, **109**, 1300 (1972).
- ⁷ Sachs, D. H., Schechter, A. N., Eastlake, A., and Anfinsen, C. B., *Biochemistry*, **11**, 4268 (1972).
- ⁸ Sachs, D. H., Schechter, A. N., Eastlake, A., and Anfinsen, C. B., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3790 (1972).
- ⁹ Anfinsen, C. B., *Science*, **181**, 223 (1973).
- ¹⁰ Scheraga, H. A., in *Current topics in biochemistry* (edit. by Anfinsen, C. B., and Schechter, A. N.), 1-42 (Academic Press, New York, 1974).
- ¹¹ Phillips, D. C., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 484 (1967).
- ¹² Hamlin, J., and Zabin, I., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 412 (1972).
- ¹³ Margoulies, M., and Greenwood, F. C., (ed.) *Structure-activity relationships of protein and polypeptide hormones* (Excerpta Medica, Amsterdam, 1972).

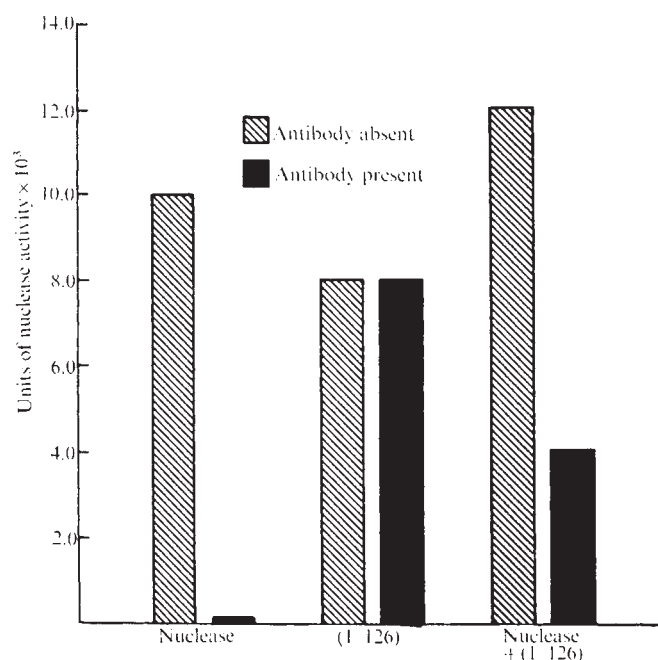


Fig. 3 Inhibition of enzymatic activity by anti-(127-149)_x antibodies: The first two pairs of bar graphs illustrate samples of nuclease (12.5 ng) or (1-126) (5 μg) containing comparable levels of enzymatic activity which were incubated with either saline (control) or with anti-(127-149)_x antibodies (5 μg), and where then assayed for residual enzymatic activity. The final pair of bar graphs show the effect of the same amount of antibody on the activity of a mixture of one-half of each of the previous amounts of nuclease and of (1-126). The increased activity of the mixture over that expected from the components may indicate that the complementary sequence of nuclease can affect the folding equilibrium of (1-126), perhaps similar to the folding 'induced' by the fragment (99-149)⁴. Antibody reduced the activity of the mixture to a level compatible with the amount of (1-126) in the mixture.

in terms of ϕ and ψ angles have been demonstrated at the level of tripeptides¹⁰, and the suggestion that proteins may fold sequentially as they are assembled at the ribosome¹¹ has been discussed widely. The demonstration that antibodies to β -galactosidase can bind to incomplete, ribosome-bound protein fragments has been taken as support for this hypothesis¹². But, the apparent requirement of nearly the entire sequence of most proteins before a significant amount of spontaneous folding occurs has been interpreted as evidence for the importance of long-range tertiary interactions in the folding process³.

The two hypotheses are not necessarily contradictory if one considers polypeptide chains to be undergoing con-

Haemoglobin Icaria, a new chain-termination mutant which causes α thalassaemia

THE human haemoglobin (Hb) variant Constant Spring (Hb CS) occurs in a number of racial groups and is involved in the aetiology of as many as 50% of all cases of Hb H disease, a disorder which presents a major public health problem in south-east Asia^{1,2}. It has an elongated α chain of 172 residues which has probably arisen by mutation of the normal α -chain-terminating codon (UAA) at position 142 to a glutamine codon (CAA), allowing read-through into extrastructural codons at the 3'-end of the α mRNA³. We report here the characterisation of Hb Icaria, a second Hb with an elongated α chain of 172 residues. Its structure is identical with that of Hb CS except that residue α 142 is lysine instead of glutamine.

The variant, which comprised 0.8% of the total Hb, was discovered during the haematological investigation of a woman from the Aegean island of Icaria. Examination of a haemolysate on starch-gel electrophoresis at pH 8.5 showed two minor slow-moving haemoglobin components, one cathodal and the other anodal. Comparison with a haemolysate containing Hb CS showed that both minor bands in the Hb Icaria sample were more cathodal than the corresponding bands of Hb CS (Fig. 1).

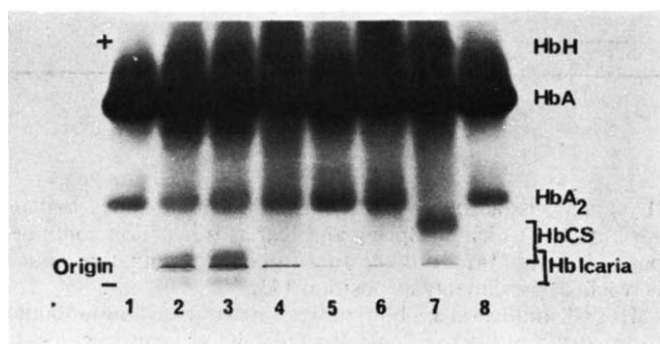


Fig. 1 Starch-gel electrophoresis (Tris-EDTA-borate system, pH 8.5, benzidine stain) of red-cell lysates prepared from the following (left to right): 1, normal adult; 2 and 3, Hb Icaria heterozygote; 4 and 5, normal adult; 6, Hb CS heterozygote; 7, individual with Hb H disease and Hb CS; 8, normal adult.

The minor components of Hb Icaria were purified on Amberlite IRC-50 by a procedure similar to that used for the isolation of Hb CS (ref. 3); they remained tightly bound to the top of the column after the elution of Hb A at 25°C with Developer 2. Hb CS, by comparison, remained on the column as a more diffuse band. Both haemoglobins were eluted as a sharp zone by increasing the salt concentration of the Developer 2 buffer to 0.5 M with NaCl. Starch-gel electrophoresis of the purified haemoglobins revealed two major slow-moving components, together with variable amounts of contaminating Hb A. In some preparations it seemed that considerable degradation of the Hb Icaria had taken place, since the mobilities of the slow-moving components were more anodal than in the original haemolysate. This behaviour is similar to that seen occasionally during the purification of Hb CS, and is attributed to degradation of the α -chain 'tail' by red-cell proteases during the purification procedure³.

A sample of purified Hb Icaria, containing about 30% Hb A, was converted to globin and fractionated by chromatography on CM-cellulose in 8 M urea at pH 6.8 (ref. 4). Two major basic α -chain components were observed in addition to the α (A) chain from the contaminating Hb A. Compared with the elution pattern from a sample made from impure Hb CS it was obvious that the minor α -chain components of Hb Icaria were relatively more basic than those of Hb CS, reflecting the

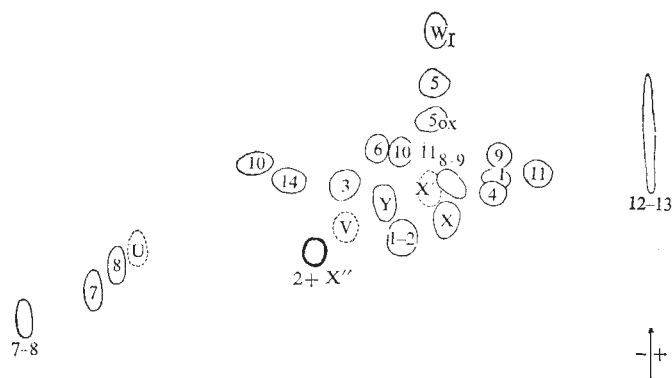


Fig. 2 Superimposed tracings of peptide maps of α (CS) and α (Icaria). Peptides common to both α chains are drawn in solid lines, those unique to α (Icaria) are dotted.

differences seen on starch-gel electrophoresis. The abnormal α -chain fractions were combined and recovered by dialysis against 0.5% HCOOH and freeze drying, and then digested with trypsin. Peptide maps of the digest were prepared by high-voltage electrophoresis at pH 4.7, followed by chromatography in *n*-butanol:acetic acid:water:pyridine (15:3:12:10 by volume) (ref. 4). A digest of authentic α (CS) chain was also run as a control. Comparison of the two (Fig. 2) revealed that all the normal α (A)-chain peptides were present and that of the three major peptides unique to the α (CS)-chain³, peptides Y and W₁ (that is, those representing residues 154 to 172) were present on the α (Icaria)-chain chromatogram, and had compositions identical to the equivalent peptides eluted from the α (CS)-chain peptide map. The remaining peptide X' (X in the α (CS)-chain digest, corresponding to residues 142 to 153) was displaced on the α (Icaria)-chain peptide map, being slightly more cathodal on electrophoresis and faster in chromatography. In addition, two new spots, U and V, were observed in the α (Icaria)-chain digest. V had the same mobility in electrophoresis as α 3 but was slower in chromatography, while U was slightly slower in electrophoresis than the free lysine (α 8). It was also observed that α 2, which normally gives a brown/grey colour with ninhydrin, was stained blue.

Peptides X', V, U and ' α 2' were cut out and the paper pieces washed with acetone before the peptides were eluted with 6 N HCl. One tenth of each sample was removed, dried and reacted with dansyl chloride for determination of the N-terminal residue⁵ and the remainder was hydrolysed for 16 h for amino acid analysis. The results are summarised in Table 1.

X' has a composition similar to α (CS)X, except for the absence of the glutamine N-terminal residue and the presence of N-terminal alanine. Peptide U, which has N-terminal tyrosine, has the composition of α (A)14 (Tyr.Arg) plus an additional lysine residue. ' α 2' is obviously a mixture of genuine α 2 (Thr.Asn.Val.Lys), and a peptide(X'') of composition Ser, Pro₂, Gly, Ala₁, Val₂, Arg, Lys; that is, X' + N-terminal lysine. These data are consistent with a sequence ... Tyr.Arg.Lys.Ala... for residues 140-143 (Table 1) instead of the ... Tyr.Arg.Gln.Ala... characteristic of all the examples of Hb CS yet studied^{2,3}. (Hb CS is found occasionally in Greece and was originally called Hb Athens²; we have redetermined the structure of Hb Athens and confirmed that its structure is identical with Hb CS.) Furthermore, peptide V has a composition and N-terminal residue consistent with the sequence of residues 142 to 147 (Table 1). In view of the extensive degradation of some of the finally purified samples of Hb Icaria, it seems likely that this represents a truncated version of the α (Icaria) chain, comparable to the CS2 and CS3 derivatives³ of Hb CS which are thought to be produced by proteolysis during purification of the haemoglobin.

Table 1 Amino acid compositions and sequences of α (Icaria)-chain peptides

α(Icaria)-chain peptides					αCS-chain peptides	
	X'	U	V	'α2'* (α2+X'')	α2	α(CS)X
Lys		(0.9) 1	(0.7)†1	(1.7)†2	1	
His						
Arg	(0.8) 1	(0.9) 1		(0.9) 1		1
Asx				(1.0) 1	1	
Thr				(0.9)†1	1	
Ser	(1.0) 1		(0.8) 1	(0.9) 1		1
Glx						1
Pro	(1.7) 2			(1.5) 2		2
Gly	(1.0) 1		(1.0) 1	(1.1) 1		1
Ala	(3.6)†4		(2.0) 2	(4.0) 4		4
Cys						
Val	(1.9) 2		(0.7) 1	(3.0) 3	1	2
Met						
Ileu						
Leu						
Tyr		(0.5)†1				
Phe						
Trp						

* Corrected for ratio of X'' to α_2 of 1.3/1.

† Yield unreliable due to reaction of N-terminal with ninhydrin.

There is now good evidence that human α mRNA has a stretch of at least 100 nucleotides between the chain-termination codon and the poly(A) tract at the 3'-end³. From a comparison of the amino acid sequences of Hb Constant Spring³ and Hb Wayne⁶ (a frame-shift mutant with an elongated α chain of 146 residues) a unique sequence of 26 nucleotides for codons 140-148 can be deduced for α -chain mRNA in which the normal chain-termination codon at position 142 is UAA (Fig. 3). A single base substitution in this codon to CAA (Gln) or AAA

(Lys) can thus account for the formation of elongated α -chain variants like Constant Spring and Icaria; translation continues beyond codon 141 until the first in-phase termination codon is reached, presumably at position 173.

Hb CS and Icaria are both present in extremely low amounts, of the order of 0.5 to 1%, in the red cells of heterozygotes. In the case of Hb CS, biosynthetic experiments have shown that this is unlikely to be due to a translational defect (such as a rate-limiting step at the glutamine-142 codon) during assembly

α ICARIA	Thr	Ser	Lys	Tyr	Arg	Lys	Ala	Gly	Ala	Ser	Val	Ala.....Glu
α A	Thr	Ser	Lys	Tyr	Arg							
α CS	Thr	Ser	Lys	Tyr	Arg	Gln	Ala	Gly	Ala	Ser	Val	Ala.....Glu
	AC ^U _{CAG}	UC[U]	AAA	UAC	CGU	A ^U _{AAC}	GCU	GGA	GCC	UCG	GUA	GCA.....
	AC ^U _{CAG}	UCA	AAU	ACC	GUU	AAG	CUG	GAG	CCU	CGG	UAG	CA.....
α WAYNE	Thr	Ser	Asn	Thr	Val	Lys	Leu	Glu	Pro	Arg		
	137	138	139	140	141	142	143	144	145	146	147	148.....172

Fig. 3 Partial amino acid and nucleotide sequences for α chains of haemoglobins A, CS, Icaria and Wayne. The base sequences for codons 138 and 139 have been chosen arbitrarily. Deletion of any one of the four bases before codon 140 produces the nucleotide sequence which defines the amino acid sequence at the C-terminal end of the α chain of Hb Wayne. In this diagram the third base of the codon for Ser 138 has been deleted; the base sequence of the α mRNA is thus uniquely defined from codon 140 to the second base of codon 148.

of the α (CS) chain³. Rather the evidence suggests that α (CS) mRNA may be much more unstable than normal α (A)mRNA since its activity in the cell cytoplasm seems to be dependent on continued nuclear mRNA synthesis (to be published).

Laux *et al.* have recently demonstrated the potential existence of a large base-paired loop, which includes the chain-termination codon, in human α mRNA⁷. In phage and ribosomal RNAs such double stranded regions are thought to contribute to the overall stability of the RNA molecules^{8,9}. Hbs CS, Wayne and Icaria are all produced in very low amounts and are exceptional in that they share the property that they arise by the translation of stretches of mRNA that normally remain untranslated. If these regions of the mRNA are essential for its stability or functional integrity, then their unfolding during translation may render the α mRNA more susceptible to degradation by nuclease attack, thus leading to low levels of functional mRNA.

There is now strong genetic and biosynthetic evidence that the Hb CS mutation results in an overall deficit of α chains in affected red cells¹⁰, and that its interaction with the severe α -thalassaemia gene (α -thal₁) causes about half the cases of Hb-H disease in south-east Asia^{2,11}. Preliminary studies indicate that Hb Icaria also produces a disorder similar to α thalassaemia. The discovery of a further chain-termination variant causing α thalassaemia suggests that inefficiently synthesised Hb variants of this type may account for other forms of thalassaemia. Obviously the blood of all thalassaemic patients should be screened carefully for trace amounts of haemoglobin variants.

J.B.C. and D.J.W. are grateful to the Wellcome Trust and the Medical Research Council for financial support.

J. B. CLEGG
D. J. WEATHERALL

Department of Haematology,
University of Liverpool,
Liverpool L69 3BX, UK

I. CONTOPOLU-GRIVA
K. CAROUTSOS
P. POUNGOURAS
H. TSEVENIS

14 Rue Vassilissis Sophias,
Athens (138) Greece

Received May 7, 1974.

- ¹ Milner, P. F., Clegg, J. B., and Weatherall, D. J., *Lancet*, i, 729 (1971).
- ² Fessas, P., Lie-Injo, L. E., Na-Nakorn, S., Todd, D., Clegg, J. B., and Weatherall, D. J., *Lancet*, i, 1308 (1972).
- ³ Clegg, J. B., Weatherall, D. J., and Milner, P. F., *Nature*, **234**, 337 (1971).
- ⁴ Clegg, J. B., Naughton, M. A., and Weatherall, D. J., *J. molec. Biol.*, **19**, 91 (1966).
- ⁵ Woods, K. R., and Wang K-T., *Biochim. biophys. Acta.*, **133**, 379 (1967).
- ⁶ Seid-Akhavan, M., Winter, W. P., Abramson, R. K., and Rucknagel, D. L., *Blood*, **40**, 927 (1972).
- ⁷ Laux, B., Dennis, D., and White, H. B., *Biochem. biophys. Res. Commun.*, **54**, 894 (1973).
- ⁸ Fellner, P., Ehresmann, C., Stiegler, P., and Ebel, J.-P., *Nature new Biol.*, **239**, 1 (1972).
- ⁹ Min Jou, W., Haegeman, G., Ysebaert, M., and Fiers, W., *Nature*, **237**, 82 (1972).
- ¹⁰ Clegg, J. B., and Weatherall, D. J., *Ann. N.Y. Acad. Sci.* (in the press).
- ¹¹ Wasi, P., Na-Nakorn, S., Pootrakul, S., and Panich, V., *Ann. hum. Genet.*, **35**, 467 (1972).

Measurements of unwinding of *lac* operator by repressor

We report here measurements of the rates of association and dissociation of the *lac* repressor and covalently closed λ *plac* DNAs of various degrees of superhelicity, from which we deduce that the binding of repressor unwinds the *lac* operator by 40° to 90°. This small unwinding angle is inconsistent with

binding mechanisms involving considerable disruption of DNA base-pairs and, in particular, with the model first proposed by Gierer¹ for specific repressor-operator interactions. Our measurements were prompted by the existence of a twofold symmetry in the base sequence of the *lac* operator, which is a prerequisite of the Gierer model. The minimal base sequence of the *lac* operator, determined by Gilbert and Maxam², is depicted in Fig. 1 as a regular double helix (Fig. 1a), and as a Gierer-type structure (Fig. 1b). According to Gierer's model, the *lac* repressor interacts specifically with the hairpinned structure shown in Fig. 1b.

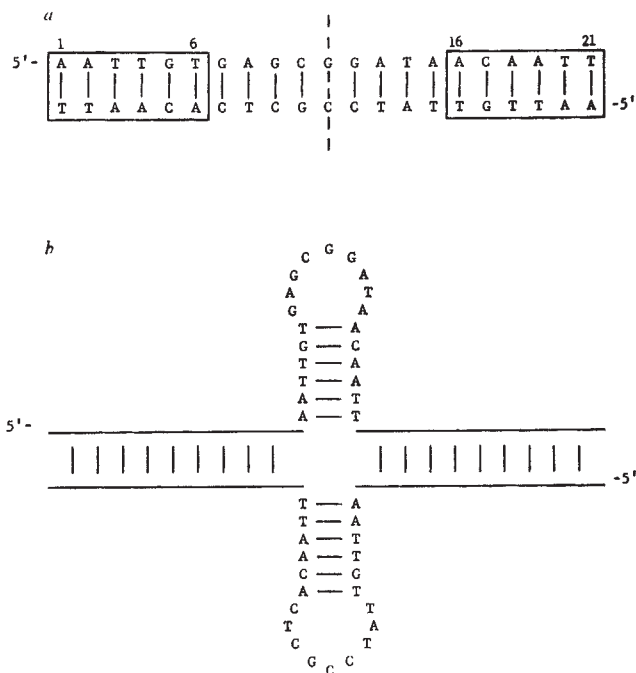


Fig. 1 a. The minimal base sequence of the *lac* operator, taken from ref. 2. The boxed regions are the symmetrical hexameric sequences which form the hydrogen-bonded regions of the two stems shown in b.

The conversion of the *lac* operator from the structure shown in Fig. 1a to the one shown in Fig. 1b reduces the number of helical turns between the two complementary strands by approximately two full turns. If the twofold symmetry in base sequence extends beyond the published sequence for the operator², the short stems shown in Fig. 1b could be lengthened further, with an even greater reduction in the number of helical turns between the complementary strands. For a negatively superhelical DNA, a reduction in the number of helical turns between the complementary strands, which decreases the absolute number of superhelical turns, is favoured thermodynamically³. Thus for the binding of the *lac* repressor to two otherwise identical DNAs containing the operator, but with only one of the two DNAs containing negative superhelical turns, a direct prediction from the Gierer model is that the affinity of the repressor to the negatively superhelical DNA should be orders of magnitude higher than that to the DNA without superhelical turns⁴. Maniatis and Ptashne⁵ have used this approach to study the binding of the λ repressor to its operators, and have concluded that it is highly unlikely that the λ operators form some structure other than a linear duplex.

Techniques have been developed for studying the specific interaction between the *lac* repressor and operator^{6,7}. The binding constant can be obtained from either equilibrium⁶ or kinetic measurements^{7,8}. The high precision of the kinetic method makes it the method of choice in determining whether the binding of the *lac* repressor significantly unwinds the operator.

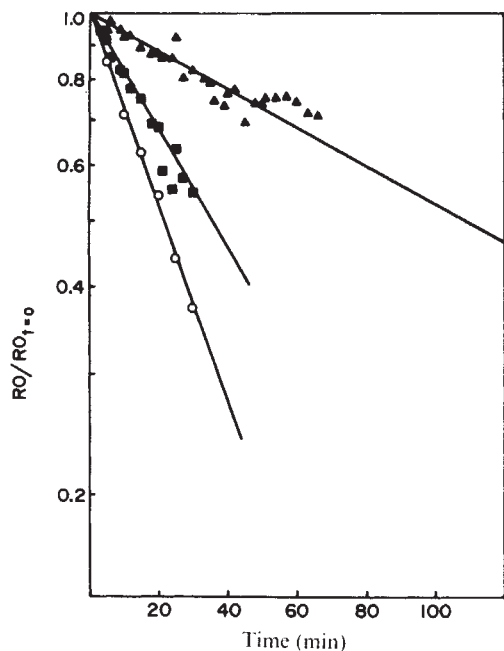


Fig. 2 Rates of dissociation of repressor-operator complexes formed with operator DNAs with various degrees of superhelicity. The *lac* repressor was isolated from an *E. coli* strain, BMH461 (ref. 9), carrying a mutation (i^q), which leads to overproduction of the repressor. The purification procedure (to be published elsewhere), which includes DNA-cellulose chromatography, yields consistently, very pure and active repressor. The DNA was extracted from the λ plac5 phage purified from a low-thymine-requiring lysogenic strain MBC5 (*(lac pro_{AB})* $\Delta_{XIIIT}B^{-1}$ $\Delta_{I}naIAR$ (λ C₁₈₅₇S₇ *plac5* $i^{-o^{+}z^{+}y^{-}}$) after growth in the presence of 3H or ^{14}C -labelled thymine. The cyclisation and the covalent closure of the DNA in the presence of various amounts of ethidium bromide were done according to published procedures¹⁰. The purified covalently closed DNA samples were used within a few days. Fifty per cent of the DNA molecules of a 3H -labelled sample with a specific activity of 2×10^5 c.p.m. μg^{-1} for example, were spontaneously converted to the nicked form by radiolysis in about 2 weeks. The membrane filter assay for repressor-operator complexes (RO) was used to titrate the repressor solution⁶ as well as to measure the kinetic constants^{7,8} of the repressor-operator interaction. The binding buffer used for all assays contained 0.01 M Tris buffer, pH 7.4, 0.01 M MgCl₂, 0.01 M KCl, 0.0001 M EDTA, 0.0001 M dithiothreitol, 5% dimethylsulphoxide and 25 μg bovine serum albumin per ml, which had been heated at 60°C for 30 min in 0.01 M Tris, pH 9, and then neutralised. The filtering buffer had the same composition as the binding buffer, but without the bovine serum albumin and dimethylsulphoxide. The purified repressor and the bovine serum albumin were shown to contain negligible amounts of nuclease activity. Concentrations of DNA of from 0.6 to 3×10^{-12} M operator were used in dissociation kinetic experiments, depending on the specific activity of the DNA preparation. The half-life of repressor-operator complexes is, however, independent of concentration⁷. A fourfold excess of repressor over operator was added to the DNA in the binding buffer and the repressor-operator complexes were allowed to form during a 30-min incubation at room temperature. The dissociation reaction was initiated by addition of unlabelled operator DNA (linear λ 080 *d*lac DNA) in a 100-fold excess over the labelled operator DNA concentration, and three 0.5 ml samples were filtered on HA Millipore filters, at appropriate times. The filters were washed once with 0.5 ml of filtering buffer, dried, and counted in a scintillation counter. The values obtained for the c.p.m. retained on triplicate filters were averaged. A background value of the amount of radioactivity retained on the filters in the presence of 10^{-3} M isopropyl- β -D-thiogalactoside, an inducer of the *lac* operon, was measured in each experiment and subtracted. The data are presented as the fraction of the amount of repressor-operator complex (RO) present before the addition of the unlabelled operator DNA. The data shown represent averaged values obtained in several independent experiments for each DNA. The initial slopes of the RO complex decay curves were calculated by least-square fitting of the data. $\circ$, $\sigma = -0.001$, average of four experiments $t_{1/2} = 2.2$ min.; $\blacksquare$, $\sigma = -0.017$, average of four experiments $t_{1/2} = 35$ min.; $\blacktriangle$, $\sigma = -0.035$, average of five experiments $t_{1/2} = 105$ min.

The rates of dissociation (k_b) and association (k_a) have been measured for the binding of the *lac* repressor to five covalently closed circular λ plac DNA samples with superhelical densities σ (defined as the number of superhelical turns per ten base pairs³ and based on an unwinding angle of 12° for ethidium) -0.001 , -0.017 , -0.028 , -0.035 and -0.049 (or 5, 80, 160 and 230 negative superhelical turns respectively) under the binding conditions. Figure 2 illustrates the results for the rate of dissociation of the repressor from operator DNAs with -5 , -80 and -160 superhelical turns. The negative superhelical turns of the DNA clearly stabilise the repressor-operator complex. The half-life of the complex is 22 min for the essentially untwisted DNA (-5 superhelical turns) and 105 min for the DNA with -160 superhelical turns. Results obtained for all DNA samples are summarised in Table 1.

Measurements of k_a are somewhat less accurate. Representative data are illustrated in Fig. 3. A small increase is observed with increasing negative superhelicity. Results obtained for all DNA samples are presented in Table 1.

Table 1 Affinity of the *lac* repressor for operator DNAs with various degrees of superhelicity

σ	$t_{1/2}$ (min)	$k_b \times 10^4$ (s ⁻¹)	$k_a \times 10^9$ (mol ⁻¹ s ⁻¹)	$K_a \times 10^{-13}$ (mol ⁻¹)	K_a^σ / K_a°	θ (degrees)
Linear	25	4.7 ± 0.3	2.4 ± 0.5	0.5		
-0.001	22	5.4 ± 0.2	3.4 ± 0.6	0.6		
-0.017	35	3.3 ± 0.2	5.7 ± 0.5	1.7	2.8	43
-0.028	65	1.8 ± 0.3	4.4 ± 1.1	2.5	3.9	35
-0.035	105	1.1 ± 0.3	9.2 ± 1.0	8.5	13.6	53
-0.049	78	1.5 ± 0.1	7.5 ± 1.0	5.1	8.7	31

The half-lives ($t_{1/2}$) of the different repressor-operator complexes were measured as described in the legend to Fig. 2. The rate constant of dissociation (k_b) is related to $t_{1/2}$ by the relationship $k_b = 1.17 \times 10^{-2} t_{1/2}^{-1}$, where $t_{1/2}$ is expressed in min. The rate constants of association (k_a) of the repressor to the different operator DNAs were measured as described in the legend to Fig. 3. The values shown represent the mean value of N experiments and the errors were calculated as $E = 1/N \sqrt{\sum (X - \bar{X})^2}$ where $X - \bar{X}$ is the deviation from the mean for each experiment. In general $N = 4$ except in the cases of the measurement of k_b with the DNA of $\sigma = -0.035$ ($N = 5$) and of k_a with the DNA of $\sigma = -0.035$ ($N = 5$) and of k_a with the DNAs of $\sigma = -0.017$ ($N = 2$), $\sigma = -0.035$ and $\sigma = -0.049$ ($N = 3$). The uncoiling angle of θ of the DNA helix due to the binding of a *lac* repressor is calculated from the equation¹

$$K_a^\sigma / K_a^\circ = \exp(-1/RT (dG_T/d\tau) (\theta/360))$$

where K_a^σ and K_a° are the equilibrium constants for the binding of the repressor to DNAs with superhelical densities σ and σ° respectively, and $dG_T/d\tau$ is the free energy change per unit change in τ , the number of superhelical turns. The recent unpublished results of Hsieh and Wang, $dG_T/d\tau = 505$ RT σ , were used in the calculation of θ . Previously Bauer and Vinograd³ gave $dG_T/d\tau = RT(844\sigma - 2640\sigma^2)$. If the results of Bauer and Vinograd are used, the calculated θ values are lowered by approximately a factor of 1.5.

The affinity of the repressor for the operator, $K_a \equiv k_a/k_b$, increases with increasing negative superhelicity up to a factor of approximately 14 for the DNA with -160 superhelical turns. Its affinity for the highly superhelical DNA with -230 turns is somewhat lower than for the DNA with -160 turns. The reason for this decrease is not clear. It has been observed¹² that for highly twisted PM2 DNAs with a negative superhelical density greater than ~ 0.045 , the template activity of the DNA for transcription by *E. coli* RNA polymerase also decreases with increasing negative superhelicity. It is plausible that for such highly twisted DNAs, in addition to the free energy of superhelix formation³, other factors such as steric hindrance, change of hydration of the DNA helix, and structural distortion of the helix¹³ are also affecting the interactions between the DNA and other macromolecules.

From the dependence of the equilibrium constant K_a on σ , the unwinding angle θ of the *lac* operator due to the binding of

a repressor can be calculated from the free energy of superhelix formation⁴. The values are tabulated in the last column of Table 1. On the binding of a *lac* repressor, the operator unwinds by about 40°, or approximately one tenth of a helical turn. This small unwinding cannot be reconciled with the prediction of the Gierer model for the operator structure shown in Fig. 1*b*, unless the operator exists as the hairpinned structure in the absence as well as in the presence of repressor. The melting temperature of the operator fragment² indicates, however, that it is highly unlikely that the operator assumes the hairpinned structure in the absence of repressor.

We also tried to measure the unwinding of the *lac* operator by the method of Saucier and Wang¹⁴. No definitive conclusion was obtained by this technique, which is not unexpected since the estimated value for θ is approximately the limit of detection of this technique.

Maniatis and Ptashne⁵ have shown, by measuring the amounts of λ repressor bound at equilibrium to linear and superhelical λ DNAs, that virtually no unwinding of the λ operator occurs on binding of the λ repressor. The observed difference in affinity for the two DNAs was less than a factor of two. For superhelical λ DNA isolated from infected cells, which was used in their measurements, σ is expected to be in the range of 0.026 to 0.031 (ref. 15). If λ repressor unwinds the λ operator to the same extent as in the case of the *lac* system, the difference in affinities for the superhelical and linear λ DNA samples used would be approximately a factor of 4 (Table 1). It therefore

appears that λ repressor does not unwind the λ operator as much as the *lac* repressor unwinds its operator, if at all.

In the calculation of θ from the free energy of superhelix formation, it has been implicitly assumed that the intercalative dye ethidium unwinds the DNA helix by 12°. Recent results of one of us (J.C.W.) indicate that the unwinding angle for ethidium is likely to be significantly larger than 12° with 26° being the best estimate. This would lead to a proportionately larger value for θ of about 90°. Since the size of the *lac* operator is about 30 base pairs, an unwinding of 40° to 90° suggests a significant change of the operator structure on the binding of the *lac* repressor.

Thus the conclusions of our unwinding measurements are twofold. On one hand, our results suggest that the recognition of the operator by the *lac* repressor is primarily through interactions with groups of the bases in the grooves. Recognition either by the Gierer model or by the disruption of a segment of the base pairs, thus exposing a sufficient number of bases, is inconsistent with our results. On the other hand, the specific interaction between the operator and the repressor involves a structural change of the operator, as evidenced by the unwinding measured. This structural change is likely to be important in recognition.

This work was supported by research grants from the US Public Health Service and a career development award to S.B.

JAMES C. WANG

Department of Chemistry,
University of California,
Berkeley, California 94720

MARY D. BARKLEY

Department of Chemistry,
University of California, San Diego,
La Jolla, California 92037

SUZANNE BOURGEOIS

The Salk Institute for Biological Studies,
San Diego, California 92112

Received April 26; revised June 28, 1974.

- ¹ Gierer, A., *Nature*, **212**, 1480–1481 (1966).
- ² Gilbert, W., and Maxam, A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3581–3584 (1973).
- ³ Bauer, W., and Vinograd, J., *J. molec. Biol.*, **33**, 141–171 (1968); **47**, 419–435 (1970).
- ⁴ Davidson, N., *J. molec. Biol.*, **66**, 307–309 (1972).
- ⁵ Maniatis, T., and Ptashne, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1531–1535 (1973).
- ⁶ Riggs, A. D., Suzuki, H., and Bourgeois, S., *J. molec. Biol.*, **48**, 67–83 (1970).
- ⁷ Riggs, A. D., Bourgeois, S., and Cohn, M., *J. molec. Biol.*, **53**, 401–417 (1970).
- ⁸ Jobe, A., and Bourgeois, S., *J. molec. Biol.*, **72**, 139–152 (1972).
- ⁹ Miller, J. H., Beckwith, J., and Müller-Hill, B., *Nature*, **220**, 1287–1290 (1968).
- ¹⁰ Wang, J. C., in *Procedures in nucleic acid research* (edit. by Cantoni, G. L., and Davies, D. R.), **2**, 407–416 (Harper and Row, New York, 1971).
- ¹¹ Riggs, A. D., Newby, R. F., and Bourgeois, S., *J. molec. Biol.*, **51**, 303–314 (1970).
- ¹² Wang, J. C., *J. molec. Biol.* (in the press).
- ¹³ Maestre, M., and Wang, J. C., *Biopolymers*, **10**, 1021–1030 (1971).
- ¹⁴ Saucier, J. M., and Wang, J. C., *Nature new Biol.*, **239**, 167–170 (1972).
- ¹⁵ Wang, J. C., *J. molec. Biol.*, **43**, 263–272 (1969).

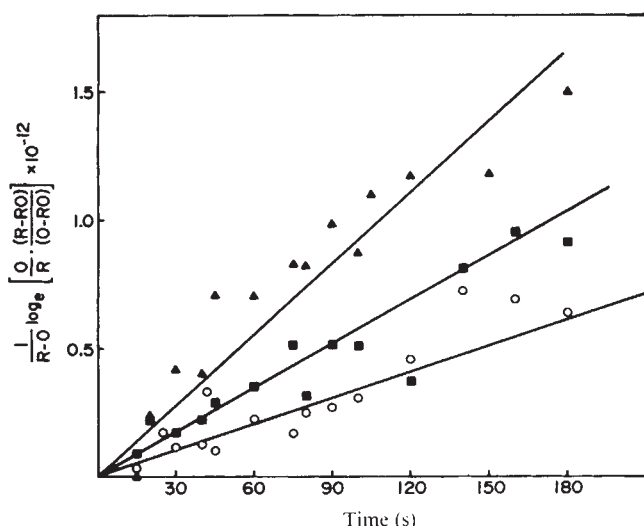


Fig. 3 Rates of association of the repressor to operator DNAs with various degrees of superhelicity. The conditions of the binding assay are the same as described in the legend of Fig. 2 except that the concentrations of the operator DNA varied from 0.4 to 1×10^{-12} M and that only a twofold excess of repressor was used. The total operator and repressor concentrations were determined precisely and the concentration of the repressor operator complex (RO) was calculated using a conversion factor estimated by filtering a known amount of RO complex under the same experimental conditions⁷. The reaction was initiated by mixing the repressor and the operator DNA and the association was stopped at appropriate times by transferring 1.5 ml samples into 100 μ l of a mixture containing 4 μ g of unlabelled ϕ X80 *dlac* DNA and 10^{-2} M *o*-nitrophenyl- β -D-fucoside. The presence of about 100-fold excess of unlabelled operator DNA prevents the formation of new labelled RO complexes while the anti-inducer, *o*-nitrophenyl- β -D-fucoside, stabilises the existing labelled RO complexes¹¹. Three 0.5-ml samples of that mixture were filtered, washed and counted and the triplicate values were averaged, as described earlier. The background value of the amount of radioactivity retained on the filters in the presence of 10^{-3} M isopropyl- β -D-thiogalactoside was subtracted from each time point. The data were plotted according to the rate equation for a bimolecular reaction⁷, with R and O denoting the total repressor and operator concentrations respectively. The rate constant of association, k_a , was obtained from the slope of the least-square line. $\circ$, $\sigma = -0.001$, average of four experiments, $k_a = 3.4 \times 10^9 \text{ mol}^{-1} \text{ s}^{-1}$; $\blacksquare$, $\sigma = -0.017$, average of two experiments, $k_a = 5.7 \times 10^9 \text{ mol}^{-1} \text{ s}^{-1}$; $\blacktriangle$, $\sigma = -0.035$, average of three experiments, $k_a = 9.2 \times 10^9 \text{ mol}^{-1} \text{ s}^{-1}$.

Subunit structure of chromatin

In the past, the structure of chromatin has remained obscure despite the efforts of many laboratories and seemed far from a simple solution¹. It has been known only that chromatin contains a repeating substructure^{2,3}. Recently, however, Kornberg proposed⁴ that chromatin structure is based on a repeating subunit of 200 base pairs of DNA and two of each of the histones (with the exception of F1

which occurs only once per subunit). Furthermore, he suggested that in chromatin these subunits form a flexibly jointed chain.

Here I present evidence in favour of this model. It is shown that 85% of chromatin can be converted to a subunit by digestion with micrococcal nuclease. This subunit contains about 205 base pairs of DNA and occurs as a discrete complex in solution. As well as demonstrating the existence of a chromatin subunit, the methods described here provide a means of isolating the subunit on a preparative scale and represent a test for the native state of chromatin.

Previous work has shown that a considerable amount of the DNA in rat liver nuclei is cleaved to integral multiples of a unit length upon digestion by an endogenous Ca^{2+} and Mg^{2+} -dependent endonuclease³. I have confirmed this result using the enzyme purified from rat liver nuclei, and demonstrated that the same DNA fragments are obtained after digestion with micrococcal nuclease (Fig. 1). A similar

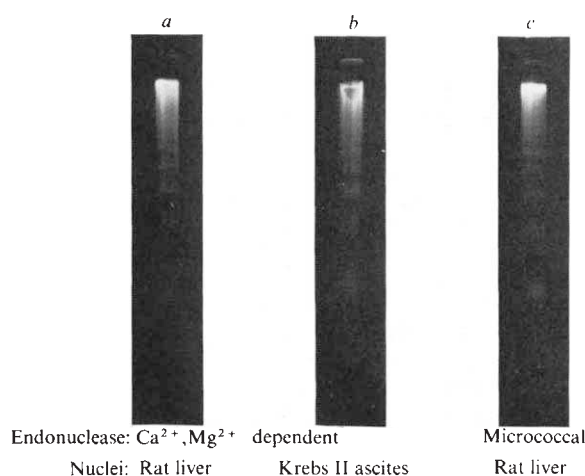


Fig. 1 Polyacrylamide gel analysis of DNA digested *in situ* with micrococcal nuclease or Ca^{2+} , Mg^{2+} dependent endonuclease. *a*, Rat liver nuclei containing the Ca^{2+} , Mg^{2+} dependent endonuclease were incubated at 1.5×10^8 nuclei ml^{-1} as described³ in the presence of 10 mM MgCl_2 and 1 mM CaCl_2 for 60 min at 37°C . *b*, Krebs II ascites nuclei which lack the Ca^{2+} , Mg^{2+} dependent endonuclease⁶ were incubated as in (*a*) in the presence of 0.5 units ml^{-1} of purified Ca^{2+} , Mg^{2+} dependent endonuclease (M. N., to be published). *c*, Incubation of rat liver nuclei with 300 units ml^{-1} of micrococcal nuclease (Worthington Biochemical Co.) in 1 mM CaCl_2 for 15 s at 37°C . After incubation the DNA was extracted, precipitated and analysed on 2.5% acrylamide gels essentially as described^{3,6}.

result is obtained with DNase I although the pattern is obscured by a very high background. Whereas both the rat liver enzyme and micrococcal nuclease require Ca^{2+} , DNase I requires only Mg^{2+} . Thus, the occurrence of regularly spaced cleavage sites is not dependent on the presence of a specific divalent cation.

Having established that readily available nucleases produce the same digestion pattern as the Ca^{2+} , Mg^{2+} dependent endonuclease, it was possible to answer many questions concerning the hypothetical chromatin subunit. For example, the proportion of chromatin based on this subunit structure could be determined. For this purpose it was necessary to determine the proportion of DNA that can be broken down into monomers (DNA of unit length associated with the hypothetical chromatin subunit of which the sizes of the larger DNA fragments [dimers, trimers, and so on] are integral multiples) or resolved multiples thereof if it is digested *in situ* with saturating amounts of the micrococcal enzyme. It is evident from Fig. 2 that nearly all DNA is converted to the monomer size. In

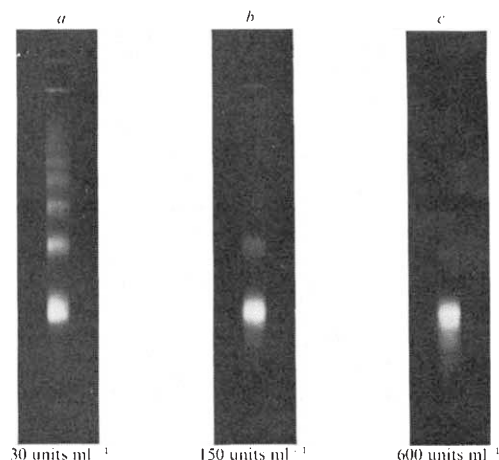


Fig. 2 Titration of chromatin digestion with micrococcal nuclease. Rat liver nuclei were incubated for 6 min at 37°C in a standard reaction mixture as described for Fig. 1c with increasing concentrations of micrococcal nuclease: *a*, 30 units ml^{-1} ; *b*, 150 units ml^{-1} ; and *c*, 600 units ml^{-1} . Incubation mixtures were processed and analysed as described in the legend of Fig. 1.

addition to the analysis of the DNA fragments on gels, the amount of TCA soluble DNA had to be taken into account in order to obtain a reliable value for the proportion of chromatin consisting of the repeating subunit. Under conditions converting all the DNA on the gel to monomers and resolved multiples of it (Fig. 2a), 13% of the DNA was acid soluble. This shows that at least 87% of the chromatin consists of the subunit, since the background of DNA between the bands is extremely low (as judged by its evaluation between the well separated first three bands). This estimate represents only a lower limit since some of the TCA soluble DNA is probably due to partial degradation of DNA in the substructure as indicated by the finding that further digestion produced more acid soluble DNA (28% in Fig. 2c).

It is evident from Fig. 2 that increasing digestion with micrococcal nuclease is accompanied by the appearance of fragments smaller than the monomer which might arise by cleavage at a few sites located within the subunit. After relatively mild digestion, on the other hand, the monomer can be observed to consist of a doublet (Fig. 3). Increasing digestion shifts the relative intensities of the two bands in favour of the lower band which suggests that it is produced from the upper band. Thus it is conceivable that the upper band is the true monomer while the lower band arises by cleavage at a preferred internal site of the monomer.

To clarify the relationship between the monomer doublet and the higher DNA bands an accurate determination of their size and breadth is required. This was achieved by calibration with a series of sequenced DNA fragments generously supplied by Dr J. W. Sedat. Thus the DNA of a micrococcal nuclease digest, the *Hin* fragments and a set of sequenced single-stranded Endo IV products of ΦX174 were run in parallel on a 99% formamide slab gel (not shown). From the sharpness of the *Hin* fragment bands it was apparent that no partial renaturation occurred on the gel. A logarithmic plot of the molecular weight of the *Hin* (P. G. N. Jeppesen, personal communication) and Endo IV (ref. 5 and unpublished results of J. W. Sedat and F. Sanger) products (the lengths of the *Hin* fragments had been determined by EM and did not deviate from their true value by more than 10% as judged by comparison with the sequenced Endo IV products) as a function of the square root of their mobility, resulted in a smooth calibration curve which was linear in the range of 200–400 bases. From comparison with this calibration curve the following values were obtained for the monomer doublet, dimer and

trimer: monomer doublet = 205 ± 15 base pairs and 170 ± 10 base pairs; dimer = 405 ± 35 base pairs; trimer = 605 ± 40 base pairs. (The breadth of the bands reflects true heterogeneity in size rather than diffusion in the gel—see also ref. 6.) The sizes of the monomer bands are consistent with an estimate of the average molecular weight of monomer DNA from sedimentation analysis on sucrose gradients⁹.

The measurements clearly show that no multiples of the lower monomer band are found and strongly suggest that both dimer and trimer correspond to twice and three times the length of the upper monomer band rather than a linear combination of both monomer bands. The data, therefore, favour a model in which the monomer doublet consists of the real monomer (205 base pairs) and a degradation product of it (170 base pairs) produced by cleavage at a site located approximately 20% within the monomer. No 'monomers', however, are observed arising from cleavage at this site in the dimer, that is, the monomer has never been observed as a triplet (with a third band of 240 base pairs). A possible explanation would be that the internal cleavage site only becomes accessible in the free subunit.

The results presented so far concern primarily the composition of the chromatin subunit. Evidence that the subunit occurs as a discrete complex in solution comes from sucrose gradient analysis of chromatin previously digested with micrococcal nuclease. As demonstrated in Fig. 4 a regular series of seven resolved peaks is observed which have been shown to contain DNA of the size of the monomer (first peak), dimer (second peak), trimer (third peak) and so on. Thus histone-histone interaction between adjacent subunits is not sufficient to hold them together. Since the sucrose gradient is isokinetic (for a particle density of 1.5 g cm^{-3}) the S-values are proportional to the

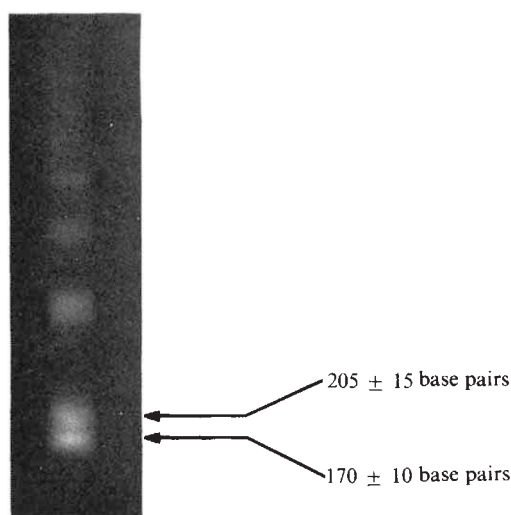


Fig. 3 Monomer doublet after 'mild' micrococcal nuclease digestion. After incubation of rat liver nuclei with micrococcal nuclease (15 s at 37°C , 150 units ml^{-1}) the nuclei were pelleted at low speed, resuspended in 0.2 mM EDTA, and homogenised with a Waring blender. The homogenate was centrifuged for 30 min at $12,000g$ and the supernatant containing the digested chromatin was processed and analysed as described for Fig. 1.

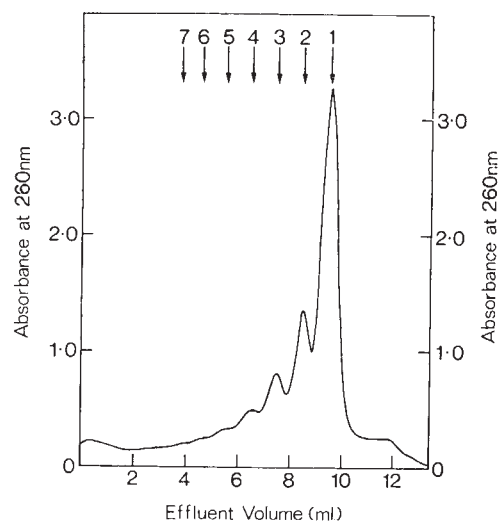


Fig. 4 Sucrose gradient analysis of digested chromatin. Digested chromatin was prepared from rat liver nuclei as described for Fig. 3 except that incubation was for 2 min rather than 15 s. The chromatin supernatant (5.5 A_{260} units) was layered on a 11.5-ml isokinetic sucrose gradient (D. Calhoun, M. N. and H. Noll, in preparation) with $c_1 = 5\%$, $c_2 = 28.8\%$, and $V_m = 55.5 \text{ ml}$ in 0.2 mM EDTA, pH 7 and centrifuged for 12.75 h at 28,000 r.p.m. and 4°C in a Beckman SW40 rotor. The gradient was monitored for absorbance by passing the effluent from the bottom of the tube through a turbulence-free flow cell (Molecular Instruments Co., PO Box 1652, Evanston, Illinois 60201, USA).

distance migrated in the gradient⁷. By calibration with 30S ribosomal subunits of *Escherichia coli*⁸ it was shown that the chromatin subunits sediment at $11.2 \pm 0.4S$ and the chromatin dimers at $15.9 \pm 0.5S$. These values have been confirmed by sedimentation velocity analysis in an analytical ultracentrifuge. The first two peak fractions when fixed with formaldehyde band both as a homogenous peak with a buoyant density of 1.45 g cm^{-3} in a CsCl gradient, exhibit a protein to DNA ratio of approximately 1.3 and contain in addition to all five major histones some non-histone proteins. Van Holde and coworkers have reported a 12S product of micrococcal nuclease digested chromatin⁹. The 12S material differs from the 11S subunit reported here in size of DNA (about 100 rather than 205 base pairs) and protein to DNA ratio (1.5 rather than 1.3). The 12S material may arise from the 11S subunit through overdigestion (ref. 4, footnote 8).

I thank Drs R. D. Kornberg, F. H. C. Crick and J. D. Smith for discussions and criticism and Drs J. W. Sedat and P. G. N. Jeppesen for Endo IV and Hin fragments. This work has been supported by a long term fellowship from EMBO.

MARKUS NOLL

MRC Laboratory of Molecular Biology,
Hills Road, Cambridge CB2 2QH, UK

Received May 30; revised July 29, 1974.

- ¹ Huberman, J. A., *Ann. Rev. Biochem.*, **42**, 355-378 (1973).
- ² Pardon, J. F., Wilkins, M. H. F., and Richards, B. M., *Nature*, **215**, 508-509 (1967).
- ³ Hewish, D. R., and Burgoyne, L. A., *Biochem. biophys. Res. Commun.*, **52**, 504-510 (1973).
- ⁴ Kornberg, R. D., *Science*, **184**, 868-871 (1974).
- ⁵ Ziff, E. B., Sedat, J. W., and Galibert, F., *Nature new Biol.*, **241**, 34-37 (1973).
- ⁶ Burgoyne, L., Hewish, D., and Mobbs, J., *Biochem. J.* (in the press).
- ⁷ Noll, H., *Nature*, **215**, 360-363 (1967).
- ⁸ Tissières, A., Watson, J. D., Schlessinger, D., and Hollingworth, B. R., *J. molec. Biol.*, **1**, 221-233 (1959).
- ⁹ Sahasrabudhe, C. G., and Van Holde, K. E., *J. biol. Chem.*, **249**, 152-156 (1974).

Protein synthesis and the release of the replicated chromosome from the cell membrane

In *Escherichia coli*, chromosome replication must be followed by a short period of protein synthesis for subsequent division of the cell¹. The protein synthesised during this period has been termed 'termination protein'^{1,2}. We have set out to test the hypothesis² that this post-replication protein synthesis is required to release the chromosomal DNA from its attachment to the cell membrane and that this detachment is necessary for subsequent cell division. The results of the experiments reported here provide support for this model.

A recently-described technique permits the isolation of the chromosome of *E. coli* as a highly folded condensed structure complexed with small amounts of RNA and protein³. Both the RNA and the protein (which is largely RNA polymerase) are required for maintaining the chromosome or 'nucleoid' in its condensed state^{4,5}. It has been hypothesised that this folded form is the normal state of the chromosome within the intact cell. When a modified extraction procedure is used, the folded chromosome can be isolated attached to fragments of the cell membrane^{4,6}. According to Worcel and Burgi⁶, the chromosome becomes detached from the membrane when replication is completed in the absence of a required amino acid, but becomes re-attached some time after the addition of the amino acid. They therefore conclude that the chromosome is attached to the membrane during replication and is released after the completion of replication. We describe here experiments which confirm these findings and also

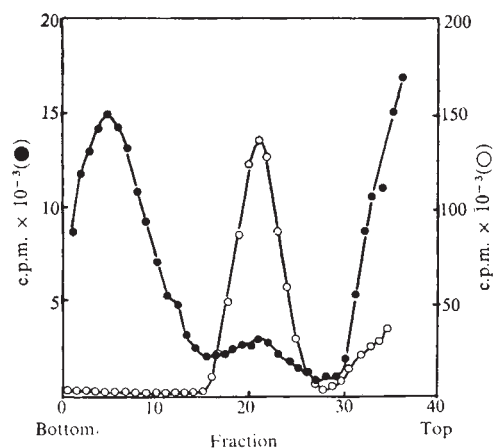


Fig. 1 Sucrose gradient profiles of cell lysates prepared at 0° C and at 25° C. ³H-thymidine (0.5 μCi ml⁻¹, 90.4 mCi mg⁻¹) was added to an exponential culture of *E. coli* Km7T⁻ growing at 37° C in 007-glucose minimal medium¹ supplemented with threonine (50 μg ml⁻¹), leucine (50 μg ml⁻¹), thiamine (10 μg ml⁻¹) and thymine (10 μg ml⁻¹). Growth was continued for 60 min and then the cells were rapidly harvested and lysed essentially as described by Stonington and Pettijohn³. The cells were harvested by centrifugation and the pellet (2–3 × 10⁹ cells) resuspended at 0° C in 0.4 ml of a solution containing 0.01 M Tris (pH 8.1), 0.01 M sodium azide, 0.1 M sodium chloride, 20% sucrose (w/v) and 0.01% diethyl-pyrocabonate (added separately for each experiment). Aliquots (0.1 ml) of a 30 mg ml⁻¹ solution of lysozyme in 0.12 M Tris (pH 8.1) and 0.05 M EDTA were immediately added and 30 s later 0.5 ml of a lysis solution containing 1% Brij-58, 0.4% deoxycholate, 2 M sodium chloride, 0.01 M EDTA (pH 8.1) was added. Lysis was allowed to continue at 0° C or 25° C for 10 min. The lysate was spun at 4,000g at 0° C for 5 min and the supernatant layered on to a 5 ml 10–30% (w/v) sucrose gradient containing 0.01 M Tris (pH 8.1), 1 M sodium chloride, 0.001 M EDTA and 0.001 M 2-mercaptoethanol. The gradients were spun in a Beckman SW50.1 rotor for 20 min at 17,000 r.p.m. and at 2–4° C. Fractions were collected on Whatman 3MM filters and the filters washed in 10% TCA (2×) and acetone, dried and counted in a toluene based scintillation fluid. ●, Cells lysed at 0° C; ○, cells lysed at 25° C.

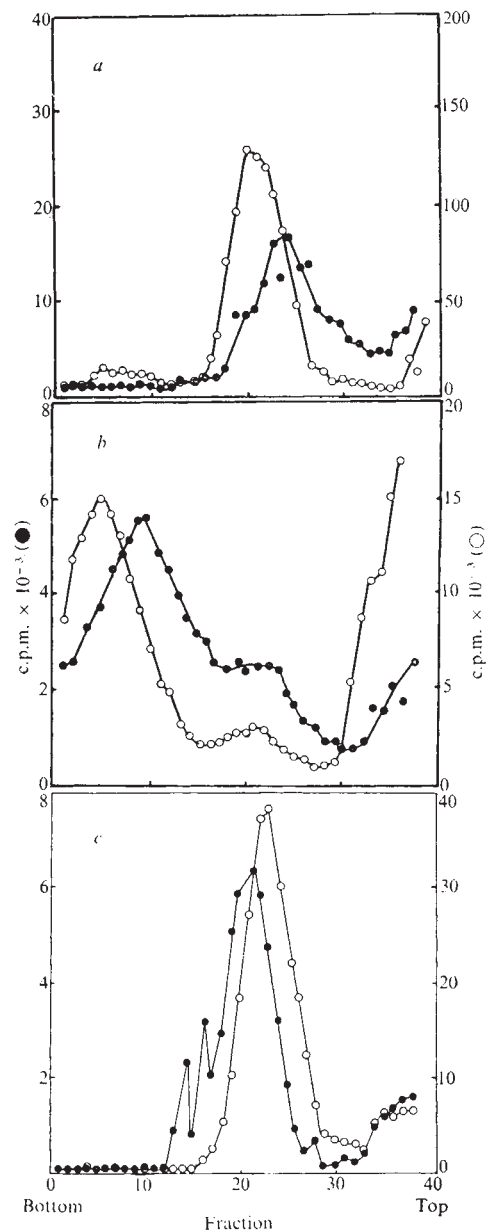


Fig. 2 Detachment of nucleoids from the membrane. ³H-thymidine (0.5 μCi ml⁻¹; 90.4 mCi mg⁻¹) was added to an exponentially growing culture of *E. coli* Km7T⁻ in supplemented minimal medium (see Fig. 1). After 60 min, cells were treated in one of three ways. *a*, Cells were incubated for 60 min with rifampicin (500 μg ml⁻¹) and then washed on a membrane filter with prewarmed medium¹, after which they were resuspended in fresh medium and nucleoids prepared at 4° C 10 min later. *b*, Cells were incubated in rifampicin and washed as in (*a*), but resuspended in medium containing chloramphenicol (160 μg ml⁻¹) before preparation of nucleoids at 0° C 10 min later. *c*, Cells were incubated for 50 min with chloramphenicol (160 μg ml⁻¹) before preparation of nucleoids at 25° C. ●, Nucleoids prepared from treated cells at 0° C (*a* and *b*) or 25° C (*c*). ○—○, Nucleoids prepared from exponentially growing cells at 0° C (*b*) or 25° C (*a* and *c*).

show that protein synthesis is required for the release of the replicated chromosome from the cell membrane.

E. coli Km7T⁻ was used in these experiments. In this strain RNA synthesis can be rapidly inhibited by the addition of rifampicin and then restarted by its removal⁷. In the presence of rifampicin (as in the absence of required amino acids) chromosome replication completes but is not reinitiated¹. It was found necessary to use rifampicin rather than amino acid starvation because the latter allowed considerable turnover of protein^{8–10}. This turnover

was found to be sufficient to allow the synthesis of termination protein (N.C.J., unpublished results).

Nucleoids were isolated from the cells by the procedure of Stonington and Pettijohn³. We found the sedimentation of these bodies on sucrose gradients to be very similar to that described by other workers^{4,6}. When exponentially growing cells were lysed at 25° C all the DNA (prelabelled with ³H-thymidine) was found in a broad, slowly sedimenting peak characteristic of membrane-free nucleoids (Fig. 1). When the cells were lysed at 0° C, however, the DNA was found to be mainly in a fast sedimenting peak characteristic of membrane-bound nucleoids (Fig. 1). In the cells lysed at 0° C, a minor fraction of the DNA sedimented at the rate expected for membrane-free nucleoids (as found also by others⁶). These may represent the chromosomes extracted from the cells which had com-

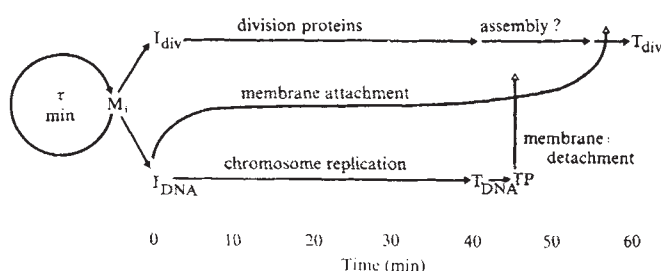


Fig. 3 Model of the cell cycle in *E. coli* (modified from Jones and Donachie¹, Fig. 6). Doubling of the initiation mass (M_i) takes place every mass doubling time (τ min). At each doubling chromosome replication (I_{DNA}) and synthesis of a set of division proteins (I_{div}) are initiated approximately simultaneously. Completion of the synthesis of division proteins takes place at about 40 min and this in turn initiates a sequence of events (IA) which takes 20 min to complete, does not require the synthesis of DNA, RNA or protein and which leads to cell division. Initiation of chromosome replication requires the attachment to a specific membrane site and this attachment prevents the completion of a terminal stage in the division sequence. Termination of DNA replication (T_{DNA}) after 40 min however, induces the synthesis of termination protein (TP) which is required for the release of the replicated chromosomes from the membrane and, consequently, for eventual cell division.

pleted their rounds of chromosome replication, as the proportion observed (about 17%) is close to the expected proportion of DNA which is in non-replicating chromosomes in an asynchronous exponential population with the observed generation time (about 60 min)¹¹.

In agreement with other workers, it was found that folded chromosomes could not be isolated from cells treated with rifampicin⁴. This is in accord with the idea that continuing RNA synthesis is necessary to maintain the nucleoid in its folded form. The chromosomes are recovered in the folded form within 10 min or less of removing the rifampicin, however, even in the presence of chloramphenicol, and so only a small amount of new RNA synthesis and no new protein synthesis is needed for refolding of the chromosome.

This enabled us to use the following procedure to test whether protein synthesis is needed for the dissociation of the chromosome from the membrane. An exponential culture was treated with rifampicin for 60 min to allow completion of all rounds of chromosome replication. The rifampicin was removed and RNA synthesis resumed for 10 min (to allow refolding of the chromosome) in the presence or absence of chloramphenicol. Nucleoids were then prepared from these cells at 0° C. If protein synthesis is necessary for detachment, then this detachment would be prevented by the chloramphenicol and so the nucleoids would remain membrane bound. The results in Fig. 2 show that this is indeed the case. The nucleoids prepared from the cells that were allowed to resume RNA

synthesis in the absence of chloramphenicol, sedimented at a position characteristic of membrane-free nucleoids (Fig. 2a). When chloramphenicol was present, however, the nucleoids prepared were found to be membrane bound (Fig. 2b). Fig. 2c shows the sedimentation coefficients of nucleoids prepared from untreated exponentially growing cells and cells treated with chloramphenicol. The results show that the chloramphenicol does cause a small increase in the sedimentation coefficient of the nucleoid¹² but this increase is not significant enough to account for the above results.

The sedimentation peaks of both the membrane-bound and the membrane-free nucleoids were slightly displaced towards the top of the gradient by comparison with the corresponding membrane-bound and membrane-free nucleoids prepared from exponential cells. This would be expected if chromosome replication were completed during the rifampicin treatment because the nucleoids should each consist of a single unreplicated chromosome while those from exponential populations should consist of chromosomes in all stages of replication and contain 1–2 chromosome equivalents of DNA⁶.

A small fraction of the nucleoids from the cells to which chloramphenicol was added after the removal of rifampicin (Fig. 2b) was membrane free. This proportion was similar to the proportion of membrane-free nucleoids prepared at 0° C from exponential cells (Fig. 1). The cells from which these were obtained had presumably completed replication before the addition of the rifampicin.

The results reported here therefore confirm those of Worcel and Burgi⁶ but in addition show that a short period of protein synthesis (10 min or less) after the completion of DNA replication is required for the release of the folded chromosome from the membrane. We have shown previously that termination protein synthesis at this same time is a necessary prerequisite for subsequent division¹. It is therefore tempting to speculate that the role of termination protein is to release replicated chromosomes from their sites of membrane attachment and that therefore it is the attachment of the chromosome to a membrane site (at initiation of DNA replication) which sets up a block to cell division. The subsequent release of this attachment, at termination of replication, would then abolish the block to division.

Figure 3 represents our present model for the control of cell division in *E. coli*, modified from that given earlier¹ to include the suggested role of termination protein.

N. C. JONES

W. D. DONACHIE

MRC Molecular Genetics Unit,
Department of Molecular Biology,
University of Edinburgh,
King's Buildings, Mayfield Road,
Edinburgh EH9 3JR, UK

Received April 29; revised June 3, 1974.

¹ Jones, N. C., and Donachie, W. D., *Nature new Biol.*, **243**, 100–103 (1973).

² Donachie, W. D., Jones, N. C., and Teather, R. M., in *Society of General Microbiology Symposium 23, Microbial Differentiation* (edit. by Ashworth, J. M., and Smith, J. E.), 9–44 (Cambridge University Press, London, 1973).

³ Stonington, G. O., and Pettijohn, D. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 6–9 (1971).

⁴ Pettijohn, D. E., Hecht, R. M., Stonington, G. O., and Stamato, T. D., *Steenbock Symposium on DNA Synthesis in vitro*, 145 (University Park Press, London, 1973).

⁵ Worcel, A., and Burgi, E., *J. molec. Biol.*, **71**, 127–147 (1972).

⁶ Worcel, A., and Burgi, E., *J. molec. Biol.*, **82**, 91–105 (1974).

⁷ Matzura, H., Molin, S., and Maaloe, O., *J. molec. Biol.*, **59**, 17–25 (1971).

⁸ Mandelstam, J., *Bact. Revs.*, **24**, 289–308 (1960).

- ⁹ Sussman, A. J., and Gilvarg, C., *J. biol. Chem.*, **244**, 6304–6308 (1969).
¹⁰ Brunschede, H., and Bremer, H., *J. molec. Biol.*, **57**, 35–57 (1971).
¹¹ Helmstetter, C. E., and Cooper, S., *J. molec. Biol.*, **31**, 507–518 (1968).
¹² Dworsky, P., Schaechter, M., *J. Bact.*, **116**, 1364–1374 (1973).
¹³ Donachie, W. D., *Nature*, **219**, 1077–1079 (1968).

Sodium pump stoichiometry in *Aplysia* neurones from simultaneous current and tracer measurements

THERE is growing evidence for the involvement in neuronal performance of hyperpolarising current resulting from the activities of the membrane 'sodium pump'. Studies of the stoichiometry of metabolically fuelled ionic movements^{1–6} have consistently shown that more Na is extruded than K accumulated. But while the electrical effect of such an imbalance is of immediate significance to the ongoing activity of a neurone, we are aware of only one study in which the electrogenicity of sodium pump activity in a neurone has been estimated⁷. Here we make such an estimate by comparing simultaneous measurements from a single neurone somata of tracer Na efflux and the transmembrane current attributable to that efflux. The results show that at least half of the actively extruded Na is electrogenic.

The experiments were carried out on the giant cell, 500–700 μm in diameter of the abdominal or pleural ganglions of *Aplysia californica*⁸, iontophoretically injected with ²⁴Na. The experimental arrangements are presented diagrammatically in Fig. 1.

Figure 2 shows the variations with time of the membrane potential [E_m], pump current (I), intracellular Na activity ($[\text{Na}]_i$) and ²⁴Na efflux (ϕ) following two steps of

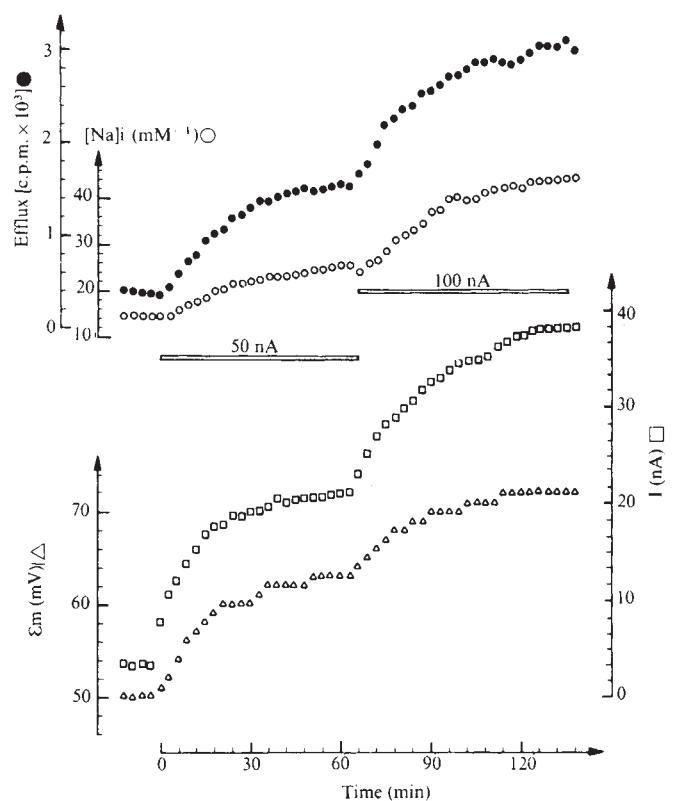


Fig. 2 Time course of membrane potential (Δ), clamp current ($\square$), intracellular Na activity ($\circ$) and efflux of ²⁴Na ($\bullet$) from a giant cell following two steps of constant ²⁴Na injection. Steady values are reached within 45 min of beginning a constant current ²⁴Na injection.

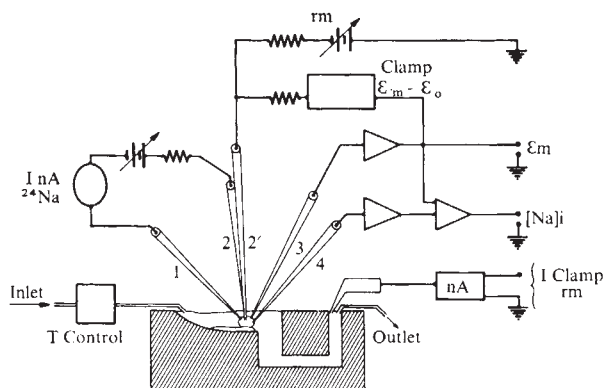


Fig. 1 The desheathed ganglion was fixed in an experimental chamber of small volume (0.25 ml) and continuously perfused at controlled temperature (normally 17° C) at a rate of 1.6 ml min⁻¹ with artificial salines. The following salines were used. Normal SW, NaCl, 452 mmol l⁻¹, KCl, 10 mmol l⁻¹, CaCl₂, 11 mmol l⁻¹, MgCl₂, 49 mmol l⁻¹, NaHCO₃, 6 mmol l⁻¹; K-free SW, same as normal SW minus KCl; Na-free (Tris) SW, Tris 513 mmol l⁻¹, KCl, 10 mmol l⁻¹, CaCl₂ 11 mmol l⁻¹, MgCl₂ 49 mmol l⁻¹; Na-free (Mg mannitol) SW, MgCl₂, 283 mmol l⁻¹, CaCl₂, 11 mmol l⁻¹, KHCO₃, 10 mmol l⁻¹, mannitol, 234 mmol l⁻¹. Controlled amounts of Na were injected into the cells between electrode 1 (2 M ²⁴NaCl solution, 15 mCi mmol⁻¹) and electrode 2 (KCl). Every 3 min, a slow time-constant voltage clamp device reset the membrane potential, E_m (measured with a separate electrode 3), to the initial value, E_o , for about 30 s by injecting a current across the membrane (electrode 2). This current was considered equivalent to the additional pump current in response to the Na injection⁷. Effluents were collected for 3 min periods and the ²⁴Na radioactivity counted. In some experiments the intracellular Na activity was measured with an Na-sensitive microelectrode (electrode 4) according to Thomas' procedure⁹.

constant and sustained injection of ²⁴Na. As soon as the ²⁴Na injection was started by passing a 50 nA current between the two injection electrodes ($t=0$ in Fig. 2) hyperpolarisation developed (lower curve in Fig. 2). Concomitantly, a positive value of pump current was recorded. After a small delay, partly due to dead space in the wash-out system, the efflux also increased. Internal Na increased and, together with the other parameters recorded, reached a new equilibrium level after 45 min constant injection. Observations such as these show that in these experiments a steady state was reached, in which Na efflux matched the injection rate. When the injection was doubled, all parameters again rose and reached higher equilibrium values.

We have used strophanthidin to define the fraction of the total ²⁴Na efflux which represents active extrusion. The data are summarised in Table 1. When strophanthidin (10^{-3} M⁻¹) was added to the perfusing saline, ²⁴Na efflux was reduced to about half, whether the saline was natural seawater (NSW) or a Na-free seawater (SW). Contrary to the effects of ionic concentration changes, the effects of strophanthidin were irreversible.

We have further characterised the components of ²⁴Na efflux by testing the effects of K-free salines, cyanide and cold. The omission of K from an otherwise normal artificial seawater decreased total ²⁴Na efflux by about 40%, but only by 15% in Na-free SW. After strophanthidin, the removal of external K had no effect on the remaining efflux. These results indicate that external K ions activate the strophanthidin-sensitive component of Na efflux⁴. Cyanide (2×10^{-3} M), perfused for as long as 1 h reduced ²⁴Na efflux no more than did strophanthidin, or if applied after strophanthidin, had no effect.

Finally, when perfusing saline was cooled to 3° C, efflux was reduced by 80%. This extreme drop in temperature

Table 1 Percentage reduction of ^{24}Na efflux

	K free	Strophanthidin (10^{-3} M)	CN $^{-}$ ($2 \cdot 10^{-3}$ M)	Cold (3° C)
Na SW	43 ± 3 (14)	56 ± 4 (9)	42 ± 5 (3)	83 ± 3 (7)
Na-free SW	15 ± 2 (9)	53 ± 3 (10)	30 ± 7 (2)	81 ± 5 (6)

Fractional inhibition of ^{24}Na efflux (mean $\pm$ s.e.m.) expressed as $(\phi_{10K} - \phi_x)/\phi_{10K} \times 100$, where ϕ_{10K} is the number of counts before changing the conditions and ϕ_x the number after that change, in NSW or in Na-free SW; changes to K-free SW, strophanthidin (10^{-3} M l $^{-1}$), CN $^{-}$ ($2 \cdot 10^{-3}$ M l $^{-1}$), or cold. Na was substituted by Tris or Mg mannitol⁹. Number of experiments in parentheses.

therefore not only decreased the strophanthidin-sensitive component of ^{24}Na efflux, but also the insensitive one.

Data obtained with internal Na-sensitive electrodes indicate that after 1 h in Na-free saline, internal Na reaches a very low level (less than 3 mM). After 1 h of iontophoretic Na injection with currents of 50–100 nA, intracellular Na activities of cells immersed in Na-free SW are steady and close to those observed in NSW, 20–30 mM. Since no entry of 'cold' Na from the bath takes place, we assume the specific intracellular ^{24}Na activity to be very much the same as that of the solution in the injecting electrode. In these conditions, the calculation of the electrogenicity of the active transport system of Na becomes possible when the strophanthidin-sensitive clamp current (that is, the amount of transferred charge) is compared with the strophanthidin-sensitive ^{24}Na efflux (that is, the total amount of Na activity extruded). It is worth emphasising that intracellular Na levels of our injected cells in Na-free SW are similar to those of uninjected cells in NSW; our analysis thus applied to Na pumping in a 'normal' range of intracellular Na.

When in Na-free (Tris) SW (Fig. 3) the efflux and clamp current reached a plateau. Introduction of strophanthidin produced a rapid, sharp reduction of both ^{24}Na efflux and clamp current to new plateau levels. The differences between the plateau values were used as measures of pump electrogenic current, I , and of active Na efflux, ϕ . In this particular experiment, the ratio $I:\phi$ had a value of 0.60. Table 2 presents data from similar experiments on six cells. Values of the electrogenic ratio vary from 0.40 to 0.65, and are clearly not related to the manner in which substitution for Na in the saline was accomplished. It

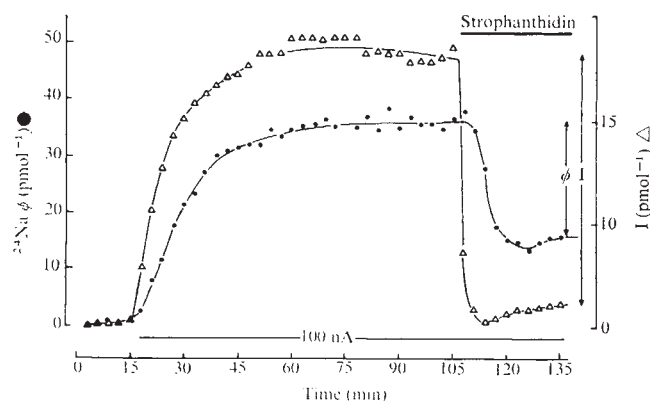


Fig. 3 Efflux and pump current during constant ^{24}Na injection of a giant cell equilibrated for 1 h and perfused with Na-free (Tris) SW: effect of strophanthidin (10^{-3} M). ^{24}Na injection (100 nA) was started about 15 min after beginning effluent collections and maintained from then on. ●, Mean flux for each 3 min collection; △ clamp current applied for 30 s at the end of each collection period. I and ϕ correspond to the strophanthidin-sensitive fraction of efflux and clamp current tabulated in Table 2.

should be emphasised that this electrogenic ratio does not depend on the degree of active transport inhibition by strophanthidin since it results from the comparison between the decreases in ^{24}Na efflux and current observed on one and the same cell. The mean figure of 0.56 taken at face value indicated that at least half of the Na actively extruded from the *Aplysia* giant neurone carries a net positive charge.

The electrogenicity and its inhibition, together with ^{24}Na efflux, by removal of external K or addition of strophanthidin, is consistent with a pump model in which extrusion of two Na^+ is balanced by the uptake of one K^+ . This 2 Na: 1 K ratio differs from the mostly evoked 3 Na: 2 K figure. In squid axon experiments, however, the data are consistent with such an exchange²⁻⁴. In human blood cells, an apparent ratio 3:2 becomes nearly 2:1, if a correction is made for ouabain sensitive K-K exchange taking place in these cells¹⁰. Our results seem to conflict more with Thomas' observations on snail giant neurones⁷. He computed that only one of three or four Na^+ extruded carried net charge.

Table 2 Clamp current (I), strophanthidin-sensitive efflux ($\text{Na}\phi$) and the ratio $I:\phi$, for experiments in Na-free SW

Na substitute	pmol min $^{-1}$ Na ϕ	I	$I:\phi$
Mg $^{2+}$, mannitol	20.8	8.6	0.41
Tris	19.8	9.9	0.50
Mg $^{2+}$, mannitol	17.0	11.1	0.65
Mg $^{2+}$, mannitol	24.0	13.2	0.55
Tris	22.0	14.0	0.64
Tris	20.7	12.3	0.60
			0.56 mean ± 0.04 s.e.m.

The discrepancy between these and our results might reflect a variation of a coupling Na:K ratio under different experimental conditions¹¹⁻¹³ (for example, differences in electrochemical gradient) or differences in properties inherent in nerve cell membranes of different species. We think that the answer to this question can be reached by further studies using our experimental approach in which ionic fluxes and their electrogenic impact can be measured simultaneously on a single neurone in different ionic and/or metabolic conditions.

We thank Dr R. C. Thomas for supplying the Na-sensitive electrodes and participating in some of the experiments in which they were used. This work was supported in part by a grant to L.T. from DGRST.

I. M. COOKE*

G. LEBLANC

L. TAUC

Laboratoire de Neurobiologie cellulaire,

CNRS and Département de Biologie,

CEN Saclay,

91190 Gif-sur-Yvette, France

*Present address: Laboratory of Sensory Sciences, 1993 East-West Road, University of Hawaii, Honolulu, Hawaii 96822.

Received March 16; revised June 27, 1974.

¹ Thomas, R. C., *Physiol. Rev.*, **52**, 563–594 (1972).² Hodgkin, A. L., and Keynes, R. D., *J. Physiol., Lond.*, **128**, 28–60 (1955).

- ³ Sjodin, R. A., and Beauge, L. A., *J. gen. Physiol.*, **51**, 152S-161S (1968).
- ⁴ Baker, P. F., Blaustein, M. P., Keynes, R. D., Manil, J., Shaw, T. I., and Steinhardt, R. A., *J. Physiol., Lond.*, **200**, 459-496 (1969).
- ⁵ Keynes, R. D., and Steinhardt, R. A., *J. Physiol., Lond.*, **198**, 581-600 (1968).
- ⁶ Garrahan, P. J., and Glynn, I. M., *J. Physiol., Lond.*, **192**, 217-235 (1967).
- ⁷ Thomas, R. C., *J. Physiol., Lond.*, **201**, 495-514 (1969).
- ⁸ Hughes, G. M., and Tauc, L., *J. exp. Biol.*, **40**, 469-486 (1963).
- ⁹ Thomas, R. C., *J. Physiol., Lond.*, **220**, 55-71 (1972).
- ¹⁰ Garrahan, P. J., and Glynn, I. M., *J. Physiol., Lond.*, **192**, 159-174 (1967).
- ¹¹ Keynes, R. D., *J. Physiol., Lond.*, **178**, 305-325 (1965).
- ¹² Adrian, R. H., and Slayman, C. L., *J. Physiol., Lond.*, **184**, 970-1014 (1966).
- ¹³ Mullins, L. J., and Brinley, F. J., *J. gen. Physiol.*, **53**, 704-740 (1969).

Evolutionary significance of autogenous regulation

GOLDBERGER¹ has drawn attention to numerous reports relating both to prokaryote and eukaryote systems in which it has been shown that an enzyme may have, in addition to its own catalytic function, a regulatory role in determining the rate of its own synthesis. He has suggested the term 'autogenous regulation' to describe such situations. The term 'autoregulation' has previously been used by others^{2,3} to describe this general phenomenon. Autogenous regulation is, however, itself an example of a more widespread phenomenon, whereby a protein may have a regulatory function as well as a catalytic or structural role. Thus for example, Slonimski and his coworkers^{4,5} have suggested that in *Saccharomyces cerevisiae*, iso-2-cytochrome *c* regulates the synthesis, not of itself, but of iso-1-cytochrome *c*, and I have proposed⁶ that in *Aspergillus nidulans*, nitrate reductase regulates not only its own synthesis, but also the syntheses of many catabolic enzymes.

In an earlier paper⁷, I speculated briefly on the possible evolutionary significance of this bifunctionality of certain proteins, suggesting that it might be a more widespread phenomenon than was at the time apparent. Now that a number of other similar examples have been reported (see ref. 1 for a list of references), it is worthwhile to develop these speculations further.

I assume that, primitively, enzyme synthesis was unregulated. For regulation to evolve, an element was required which must combine two specificities. It had to be able to interact in some way with the apparatus of gene expression to ensure that the gene product was synthesised only when the environmental conditions of the cell required it. It therefore also had to be able to recognise these environmental conditions. The evolution *de novo* of such an element, with its two unrelated specificities, would pose problems. There were already present in the cell, however, elements which had one of these functions. At this point, it is simpler to consider the evolution of inducible and repressible systems separately.

For inducible systems, the cell already possessed enzymes which had to recognise their substrates to function and it seems plausible that these would provide a starting point from which evolution could select the second specificity necessary for them to acquire additionally a regulatory function. Although, for inducible systems, an enzyme would be an ideal candidate for evolving the function of regulating its own synthesis, it is by no means the

only candidate. Any other protein which interacted either with the substrate of the enzyme whose synthesis is to be regulated, or with some metabolite whose intracellular concentration was correlated with the concentration of that substrate, would also be suitable. This may explain why the natural substrate is not the effector in some inducible systems. Examples of this are xanthine dehydrogenase I in *Aspergillus nidulans* which is induced by its product uric acid but not by its substrate hypoxanthine⁸, and the *Escherichia coli lac* system which is induced by allolactose, but not by lactose⁹.

For repressible systems, there would also be a variety of possible candidates already present in the cell, which would be suitable for selection for a regulatory role. These would include any protein which could bind either to the product of the pathway to be repressed, or to some metabolite whose concentration was correlated with the concentration of that product (for example, for some amino acid biosynthetic pathways, the charged amino-acyl tRNA). In several cases it has in fact been found that the first enzyme of the biosynthetic pathway, which recognises the end product or a related metabolite because it is subject to feedback inhibition, also has a regulatory role in determining the rate of synthesis of the enzymes of the pathway¹. These findings imply that the evolution of feedback inhibition may have predated repression.

In both inducible and repressible systems, it is possible that selection for the modification of a catalytic protein so that it acquires additionally a regulatory function, may in some cases put demands on that protein such that it could perform neither catalysis nor regulation well. In such cases, duplication of the gene specifying the protein, and subsequent divergence of the two gene copies, would allow each to be selected for one of the functions only. This would result in genes whose protein products would now seem to have only a regulatory function, but it is possible that such proteins might still contain in their amino acid sequences 'fossil' evidence of their evolutionary origin.

Beyrouther, *et al.*¹⁰ have determined the amino acid sequence of the *E. coli lac* repressor, and have found no similarities between it and those parts of the β -galactosidase sequences which are known. They argued that it was unlikely therefore that the two proteins had evolved from a common ancestor. The *lac* repressor could have evolved, however, from some other cellular enzyme which bound allolactose.

D. J. COVE

Department of Genetics,
University of Cambridge,
Milton Road,
Cambridge CB4 1XH, UK

Received June 10; revised July 25, 1974.

- ¹ Goldberger, R. F., *Science*, **183**, 810 (1974).
- ² Cove, D. J., and Pateman, J. A., *J. Bact.*, **97**, 1374 (1969).
- ³ Calhoun, D. H., and Hatfield, G. W., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2757 (1973).
- ⁴ Sels, A. A., Fukuhara, H., Pere, G., and Slonimski, P. P., *Biochim. biophys. Acta*, **95**, 486 (1965).
- ⁵ Clavilier, L., Pere, G., and Slonimski, P. P., *Molec. Gen. Genetics*, **104**, 195 (1969).
- ⁶ Cove, D. J., *Heredity*, **29**, 119 (1972).
- ⁷ Cove, D. J., *Biochim. biophys. Acta*, **113**, 51 (1966).
- ⁸ Scazzocchio, C., *Molec. Gen. Genetics*, **125**, 147 (1973).
- ⁹ Jobe, A., and Bourgeois, S., *J. molec. Biol.*, **69**, 397 (1972).
- ¹⁰ Beyrouther, K., Adler, K., Geisler, N., and Klemm, A., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 3576 (1973).

matters arising

Two-stage transformation *in vitro*

THE evidence for malignant transformation of cells *in vitro* by the combined action of benzo(a)pyrene (initiator) and phorbol ester (promoter), presented by Lasne *et al.*¹ is very striking. I feel, however, that they fail to bring out the true nature of their results.

The benzo(a)pyrene treatment of the primary rat fibroblasts was at the third passage, yet the dramatic increase in the percentage of transformed colonies was delayed until the thirty-fourth passage, when the incidence became 20.5% (benzo(a)pyrene alone) and 33.0% (with phorbol). By this time the 'primary' fibroblast culture was becoming senescent. This was indicated by the rise in frequency of transformed colonies in the controls to 11.5% by the fortieth passage.

It seems that we are not observing an increase in the rate of transformation but rather the selection of already transformed clones in a mixed population of cells. The phorbol seems to increase the selective advantage of transformed cells by 50%. There is no sign so far of two-stage transformation.

Two-stage transformation becomes apparent only when 2-d-old rats were injected with 2×10^6 cells. At the fortieth passage, although 42.5% of the cells were transformed by *in vitro* standards, they produced no tumours on injection. At the same time, cells also exposed to phorbol produced tumours in every rat. There has been a malignant transformation of the phorbol-treated cells which only becomes apparent on injection into animals.

The 'two stages' of malignant transformation are therefore, first, the transformation in colony morphology as seen *in vitro*, which is induced by benzo(a)pyrene. After 30 passages these transformed cells have a selective advantage and grow at the expense of the untransformed cells. The selective advantage is slightly enhanced by phorbol. Second, the transformation of stage 1 cells which is cryptic *in vitro*, but apparent on injection into young rats by the production of tumours. This is induced, or perhaps merely accelerated, by phorbol.

A. J. BATEMAN

Paterson Laboratories,
Manchester, UK

¹ Lasne, C., Gentil, A., and Chouroulin-kov, I., *Nature*, **247**, 490-491 (1974).

DR LASNE REPLIES—Thank Dr Bateman for the analysis of our results in our paper. I think however, that the problem is not so simple.

So far as we know, the increase in the percentage of transformed colonies (11.5% by the fortieth passage) in tissue culture, does not necessarily indicate senescence. On the contrary, it shows and confirms the beginning of an *in vitro* spontaneous cell transformation¹⁻³.

When Dr Bateman speaks about the senescent cell selection theory, he perhaps thinks of the Prehn's 'cloning selection theory'⁴. In this case, we refer him to reports⁵⁻⁷ that in chemical carcinogenesis, no selection of pre-existing transformed cells has happened.

Concerning the action of phorbol ester, and in general the action of the other promoters, on the growth of pre-existing transformed cells, we wonder whether the author is aware of studies on carcinogenesis *in vivo*. Our results are interpreted in correlation with the model established *in vivo*. In previously initiated animals, the promoter increases the tumour incidence, and shortens the latent period⁸. *In vitro*, the phorbol ester gives similar results increasing the number of transformed colonies, and accelerating the tumour development after inoculation of the cells in animals. But we have not discussed the general theory of cocarcinogenesis, which requires greater attention. Our paper merely raises this question.

Lastly, these results have been obtained and presented to the scientific public. Each investigator may present his own interpretation. We have proposed one.

¹ Earle, W. R., *J. natn Cancer Inst.*, **4**, 165-212 (1943).

² Grey, G. O., *Cancer Res.*, **1**, 737 (1941).

³ Todaro, G. J., and Green, H., *J. Cell Biol.*, **17**, 299-313 (1963).

⁴ Prehn, R. T., *J. natn Cancer Inst.*, **32**, 1-17 (1964).

⁵ Huberman, E., and Sachs, L., *Proc. natn. Acad. Sci. U.S.A.*, 1123-1129 (1966).

⁶ Mondal, S., and Heidelberger, C., *Proc. natn Acad. Sci., U.S.A.*, **65**, No. 1, 219-225 (1970).

⁷ Umeda, M., and Iype, P. T., *Br. J. Cancer*, **28**, 71-74 (1973).

⁸ Berenblum, I., and Lonai, V., *Cancer Res.*, **30**, 2744-2748 (1970).

The Musgrave Block-Amadeus Basin Complex

DAVIDSON¹ suggested that a plate tectonic model, involving continental collision, could explain the features observed in the Musgrave Block-Amadeus Basin area of central Australia. Several significant features of the area are not accounted for in his model and these are, I believe, of sufficient importance to warrant modification of the model.

In Davidson's model the Giles Complex is considered to represent part of an ophiolite thrust belt, originating in layers 2 and 3 of the oceanic lithosphere. Bearing in mind the form of ophiolite sequences, however (see, for example, the discussions at the Penrose Field Conference²), there is no indication that rocks of the Giles Complex are in fact ophiolites, even if the absence of cherts and pillow lavas in the area is ignored. There is a marked absence of olivine rich rocks, a deficiency of ultramafic rocks in general, and all parts of the Complex show some features characteristic of stratiform intrusions, such as cumulate textures, cyclic units, rhythmic layering and associated 'sedimentary type' structures^{3,4}. Repetition within the sequence of mafic-ultramafic units is largely a result of the igneous layering and cyclic units. Some intrusions in the central part of the Complex show features characteristic of crystallisation under high pressure (that is, near the base of the crust⁵) and such features are incompatible with an oceanic ophiolite type origin. Furthermore, there is evidence that the separate bodies of the Giles Complex show a depth stratification⁶ which is inconsistent with Davidson's model.

Several structural aspects and relationships are also inconsistent with that model. The bodies of the Giles Complex were intruded into granulite facies rocks—chilled margins occur in places, and unfaulted boundaries are transgressive to the earliest recognisable folds in the granulites^{4,5}. Relatively minor, local low-angle faults have affected some intrusions such as Gosse Pile⁶ and Kalka⁴. The faulting occurred under conditions of high pressure and temperature, equivalent to granulite facies⁷, and should not be confused with the much larger scale mylonitic faulting such as occurred in Woodroffe, Hinkley and Davenport. The low-angle fault-

ing was responsible for the partial dismembering of parts of the Giles Complex. But even though the felsic units of Gosse Pile were removed in this way they can be related to the more felsic Kalka intrusion which occurs to the west, along the strike of the fault, and therefore the deformation is unlikely to have caused the total removal of the supposed pillow lavas and pelagic sediments.

After the minor phase of high pressure and temperature thrusting there followed a period of regional deformation (F_2) involving both granulite facies rocks and the Giles Complex bodies (refs 3 and 4). This was followed by the intrusion of picrite plugs and olivine dolerites, and a third period of folding (F_3), before the deformation along the major E-W mylonite zones, such as the Hinckley Fault.

The eastern Musgrave Ranges had a similar, complex structural history before faulting took place along the major Woodroffe Fault and Davenport Shear⁸. That indicates that the Giles Complex had intruded granulite facies rocks and, in part, had crystallised and been deformed under conditions of high temperature and pressure and had undergone two further periods of deformation before major movement along the large thrust zones. They are therefore obviously not ophiolites.

The presence of the major thrust zones and the large volumes of mafic intrusives, indicates that this area has been one of significant tectonic activity. Apart from the exposed thrust zones and rocks of the Giles Complex, a marked gravity high extends from the Musgrave Block to the Indian Ocean. This is the Ankateell-Warri Ridge^{9,10}. These features all suggest a major uplift along the thrust zones in this area, such that upper mantle material has been brought near the surface. There is evidence to suggest that the rocks to the north of the Woodroffe Fault are of lower metamorphic grade (amphibolite facies) than those to the south (granulite or granulite-amphibolite transition facies) and also have different tectonic histories⁸.

Similarly, the Olia Gneiss containing nappe structures of Upper Proterozoic rocks in the southern Amadeus Basin (section c-d in Davidson's article) consists of amphibolite, migmatite and schist, and is not granulite facies terrain. This leads me to suspect that areas to the north and south of the major thrust zones are not as similar as they may appear to be at first, and a preconvergence period of spreading within a single continent may not be necessary.

A modified model can be proposed, in which a southern Musgrave craton collided with a northern Musgrave

(?Arunta) craton along the line now marked by the thrust zones of the Musgrave Block and by the Ankateell-Warri Ridge. The southern Musgrave craton was forced up along the thrusts, bringing to higher levels the rocks of the lower crustal granulite facies and the high pressure intrusives of the Giles Complex. Further west it did not rise so high and higher level (lower pressure) intrusives and metamorphic rocks are exposed¹¹. Because of the uplift, upper mantle material would now be nearer the surface, giving rise to the gravity anomalies. At a very high level the ophiolite complexes, folded sediments and lower grade (including blueschist) facies metamorphics would have been found, but considerable erosion has removed these, and the remnants may be found in the Upper Proterozoic Inindia and Winnalla Beds and in the Cambrian Mt Currie conglomerate, as suggested by Davidson.

The 'Woodroffe-Ankateell Suture' may thus be regarded as a major tectonic boundary and, in the same way as the Insubric Line of Europe may mark the site of a Mesozoic collision trench (for example, the Ivrea Zone¹²), the 'Woodroffe-Ankateell Suture' may mark the site of a Proterozoic collision trench. In the Australian example, however, a considerably deeper section of the boundary is exposed. If this model is correct it implies that the radiometric ages of rocks to the north of the Woodroffe Fault should be younger than those to the south.

ALAN C. MOORE

Department of Geology,
University of Cape Town,
Rondebosch, Cape 7700,
South Africa

¹ Davidson, D., *Nature phys. Sci.*, **245**, 21 (1973).

² *Geotimes*, **24** (1972).

³ Nesbitt, R. W., and Talbot, J. L., *Contr. Miner. Petrol.*, **13**, 1 (1966).

- ⁴ Nesbitt, R. W., Goode, A. D. T., Moore, A. C., and Hopwood, T. P., *Geol. Soc. S. Afr., Spec. Pub.*, **1**, 547 (1970).
⁵ Goode, A. D. T., and Krieg, G. W., *J. geol. Soc. Aust.*, **14**, 185 (1967).
⁶ Moore, A. C., *J. geol. Soc. Aust.*, **18**, 69 (1971).
⁷ Moore, A. C., *J. Petrology*, **14**, 49 (1973).
⁸ Collerson, K. D., Oliver, R. L., and Rutland, R. W. R., *J. geol. Soc. Aust.*, **18**, 379 (1972).
⁹ Lonsdale, G. F., and Flavelle, A. J., *Rep. Bur. Miner. Resour. Geol. Geophys. Aust.*, **133** (1968).
¹⁰ Rowan, I. S., *Rep. Dir. Mines Geol. S. Aust.*, **64/33** (1967).
¹¹ Horwitz, R. C., Daniels, J. L., and Kriewaldt, M. J. B., *Rep. geol. Surv. West. Aust.*, **56** (1967).
¹² Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, **75**, 2625 (1970).

Musgrave Block

MOORE'S comments require that some modification be made in the detail of the original model¹. The degree of modification, however, is not nearly as extensive as might be implied from the criticisms he presents.

His arguments are related principally to two points. First, the nature of the mafic-ultramafic rocks of the Giles Complex, their origin and mode of emplacement. Second, the time-space relationship between the colliding continental blocks. My following comments are concerned with answering objections raised by Moore in regard to these points.

The geological environment in which layered or stratiform mafic-ultramafic complexes are formed is still uncertain and it is possible that there are several such environments^{2,3}. There is definite evidence, for instance, indicating that stratiform complexes may form at spreading centres^{3,4} and thus become significant parts of the oceanic lithosphere. As such they may be later tectonically emplaced along collision boundaries. This suggestion is given further support by the presence of cumulate textured, layered mafic-ultramafic rocks within known ophiolite sequences^{5,6} (assuming ophiolites to represent ancient oceanic lithosphere). Furthermore, it has been suggested that stratiform bodies may well characterise Proterozoic ophiolites⁷. It is also significant that cumulate, layered rocks within ophiolites tend to be more deformed than other types of stratiform sequences. This is the case with the Giles Complex rocks^{7,8}.

The near parallelism of primary and tectonic layering, the close association between thrust surfaces and the layering, the presence of thrust faults at contacts with basement granulites and the gross correlation of regional trends for the mafic-ultramafic belt and the major thrust faults^{7,8}, all suggest a strong tectonic relationship between the emplacement of the Complex rocks and

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

the thrust zone. This is best explained in terms of a 'subducting oceanic lithosphere' model. In addition the marked similarity between 'alpine-type' flow layering and deformation and the tectonic layering observed in parts of the Complex^{7,8} cannot be ignored in interpreting the origin and mode of emplacement of the sequence.

The presence of granulite facies metamorphism, annealed textures and other high P/T reactions are not incompatible with the model. Subduction to depths of the order of 30 km would produce pressures and temperatures (10 kbar, 800°C) within the lithospheric slab⁹ suitable for the development of the observed phenomena¹².

The question then arises as to the order of events as recorded in the rocks, also taking into account the phases of folding that have affected the layering. The events from oldest to youngest appear to be: first, the ductile shearing, metamorphism and annealing resulting in a gabbro gneiss zone; second, the folding (F₂) of this tectonic layering⁸; third, the folding (F₃) of the Complex⁸ on a more regional scale, and fourth, brittle thrusting.

The original model again gives the best solution. The oceanic lithosphere is first underthrust to depths sufficient to produce metamorphism, annealing and so on as indicated above. Further underthrusting produces a maximum compression axis parallel to the layering and gives rise to F₂ folding¹¹. With the development of actual collision between continental blocks a period of tectogenesis and orogenesis (uplift) results in F₃ flexuring of the subducted lithosphere, slivers of which are consequently overthrust (obducted) on to the 'active' continental block¹. The latter event produces the phase of brittle faulting (major thrust faults) and mylonitisation. This sequence of events also explains the lack of a metamorphic phase in the granulites correlateable to that producing the tectonic layering in the rocks of the Complex.

Moore's comment regarding the difference in metamorphic grade in the basement rocks north and south of the thrust zone is a valid one and recent work also indicates differences in structural geometry¹². This, in fact, makes a collision model even more acceptable, as recourse to the tenuous concept of 'oscillation' is no longer required.

In conclusion, let me point out that the fundamental interpretation of the regional geology of the area by means of a plate tectonics model is still valid and is supported by other comments made by Moore in his discussion. The distribution of distinctive tectonic elements and their correlation with such a model remains the essential feature of the hypothesis. The only real alteration

is that of distinguishing as separate entities the Musgrave-Mann granulites (proto-Musgrave block) to the south and the gneiss-amphibolite terrain (Arunta? block) to the north of the thrust zone.

D. DAVIDSON

Department of Chemistry
(Geology Section)
Western Australian Institute of
Technology,
Hayman Road,
South Bentley,
Western Australia, 6102

- ¹ Davidson, D., *Nature Phys. Sci.*, **245**, 21 (1973).
- ² Wyllie, P. J., *The Dynamic Earth* (Wiley, New York, 1971).
- ³ Moores, E. M., *Earth Sci. Rev.*, **9**, 241 (1973).
- ⁴ Coleman, R. G., *J. Geophys. Res.*, **76**, 1212 (1971).
- ⁵ Moores, E. M., and Vine, J. F., *Trans. R. Soc., Lond.*, **A268**, 443 (1971).
- ⁶ Davies, H. L., *Aust. Bur. Min. Res. Bull.*, **128** (1971).
- ⁷ Moore, A. C., *J. Petrol.*, **14**, 49 (1973).
- ⁸ Nesbitt, R. W., Goode, A. D. T., Moore, A. C., and Hopwood, T. P., *Geol. Soc. S. Afr. Spec. Publ.*, **1**, 547 (1970).
- ⁹ Turner, F. J., *Metamorphic Petrology* (McGraw-Hill, 1968).
- ¹⁰ Toksoz, M. N., Mincar, J. W., and Julian, B. R., *J. geophys. Res.*, **76**, 1113 (1971).
- ¹¹ Isacks, B., Oliver, J., and Sykes, L. R., *J. geophys. Res.*, **73**, 5855 (1968).
- ¹² Collerson, K. D., Oliver, R. L., and Rutland, R. W. R., *J. geol. Soc. Aust.*, **18**, 379 (1972).

Ethylene and soil fungistasis

SMITH¹ has reported that ethylene is a causative agent of soil fungistasis, that its production in soil varies with organic matter and that in contrast with other work^{2,3} it can occur in aerobic soil conditions. His second claim is repetition of earlier work³; more recent observations⁴ suggest that soil anaerobiosis is necessary to mobilise substrates⁵ for ethylene formation by soil microorganisms.

We have attempted to reproduce Smith's experimental conditions to investigate further the effect of oxygen on ethylene formation in soil and the significance of ethylene as a fungistatic agent. The soils used, which passed through a 0.1-cm sieve, were a chalk loam (10.3% organic matter) and a sand (1.4% organic matter). The soils were packed in open glass tubes and the water tension maintained at field capacity, that is, the moisture content of soil after it had drained from a waterlogged state on filter paper overnight. The oxygen concentration in the loam was reduced from atmospheric to less than 0.1% and the ethylene concentration reached 28 v.p.m. within 2 d at 25°C. In the case of the sand,

oxygen remained at atmospheric levels after 15 d and the ethylene concentration never exceeded 0.1 v.p.m. When the experiments were repeated with soils which had been sieved to 0.2 cm instead of 0.1 cm, they did not become depleted in oxygen and no ethylene was detected. When the top of the tube was sealed, however, the concentration of oxygen decreased and ethylene was detected.

Whereas it has clearly been shown that ethylene can be fungistatic aerobically¹, it is neither fungistatic to nor metabolised by an ethylene-producing soil fungus, *Mucor hiemalis*⁶; further, there is no evidence that it is fungistatic in the soil under the conditions in which it is normally formed, that is anaerobic, since it is almost certain that anaerobiosis is itself fungistatic⁷.

Ethylene formed in anaerobic zones, however, could diffuse to and act in aerobic zones if it were not lost by further diffusion or metabolism⁸. Germinating seeds also produce ethylene⁹, presumably by an aerobic process, and this could be important in the seedling's resistance to attack by fungal pathogens.

J. M. LYNCH

S. H. T. HARPER

Agricultural Research Council,
Letcombe Laboratory,
Wantage OX12 9JT, UK

- ¹ Smith, A. M., *Nature*, **246**, 311 (1973).
- ² Smith, K. A., and Russell, R. S., *Nature*, **222**, 769 (1969).
- ³ Smith, K. A., and Restall, S. W. F., *J. Soil Sci.*, **22**, 430 (1971).
- ⁴ Lynch, J. M., and Harper, S. H. T., *J. gen. Microbiol.*, **80**, 187 (1974).
- ⁵ Lynch, J. M., *Nature*, **240**, 45 (1972).
- ⁶ Lynch, J. M., *J. gen. Microbiol.*, **83** (1974).
- ⁷ Griffin, D. M., *Ecology of Soil Fungi*, 113 (Chapman and Hall, London, 1972).
- ⁸ Abeles, F. B., Croker, L. E., Forrence, L. E., and Leather, G. R., *Science*, **173**, 914 (1971).
- ⁹ Meheriuk, M., and Spencer, M., *Can. J. Bot.*, **42**, 337 (1964).

DR SMITH REPLIES—Most of the points raised by Lynch and Harper have been clarified by our latest research¹. We have shown clearly that spore-forming anaerobic bacteria are the major producers of ethylene in soil; that ethylene is produced in anaerobic microsites in even relatively dry soil; that the anaerobic microsites result from the activity of aerobic microorganisms; and that the aerobes, in turn, are inactivated by the ethylene diffusing from the anaerobic microsites. In essence, we have described a self-regulating cycle in soil that controls microbial activity.

Other soil microorganisms, including *Mucor hiemalis*, may produce ethylene but the quantities will be limited by their own sensitivity to ethylene as

most soils are fungistatic most of the time. Also, our research has shown that ethylene production in soil is relatively insensitive to heat treatments, which rules out *M. hiemalis* as a major producer because it is killed by even mild heat treatments.

Claims that fungi are insensitive to ethylene must be interpreted carefully because in my original paper it was pointed out that the fungistatic action can be overridden by nutrients. Thus, results obtained with ethylene in pure culture cannot be extrapolated to unsterilised soil.

PMB 10, Rydalmere,
NSW 2116, Australia

¹ Smith, A. M., and Cook, R. J., *Nature* (in the press).

tRNA and ageing

HOFFMAN and McCoy¹ have reported on the nucleoside composition of tRNA from mature and old mosquitoes and mature and old mice. They reported that no alterations in the total population of tRNAs occur during ageing. The authors "point out the danger of interpreting chromatographic changes in tRNA or changes in activity of tRNA methylases in terms of the final degree of modification without actually analysing the tRNA". I could not agree with them more but write to point out that they did not analyse the tRNAs whose chromatographic profiles are changed. They analysed the crude mass of tRNAs which in metazoa may number more than 100. The isoaccepting tRNAs which differ in a variety of systems such as tumour tissue and differentiating tissue, are invariably very few in number and often they are minor species. For example, it has been shown in Ames' laboratory that in *Salmonella* tRNA^{His} is the control agent for the histidine pathway operon. In a mutant in which the operon is always open with the consequent constitutive status of the pathway, there is an isoaccepting tRNA^{His} which differs from its counterpart in the wild type by the lack of conversion of two Us to ψ s². If we assume only 64 tRNAs in *Salmonella* and an average of four ψ s per tRNA no analytical method could have established the absence of two ψ s out of 256. Under the circumstances, a mass analysis of tRNA mixtures in which the preponderance of the population has not changed is meaningless.

The senior author has shown earlier in aged mosquitoes a changed elution pattern in but five tRNAs³. Only an analysis of any of these from young and aged mosquitoes would have any meaning. This, of course, is technically

impossible at present. The authors refer to earlier work by Frazier and Yang who found no difference in elution patterns of tRNAs in young and old mouse liver⁴. An examination of that paper reveals that only nine tRNA profiles were compared and most of these were done with the RPC-2 system which is known to have inadequate resolving power for the separation of isoaccepting tRNAs. May I emphasise again to those who are not conversant with this field, that a very few, indeed sometimes but one regulatory tRNA, can have profound physiological effects. Thus, for example, Jacobson has shown that a single tRNA can control the eye colour of *Drosophila*⁵.

ERNEST BOREK

Department of Microbiology,
University of Colorado Medical
Center,
4200 East Ninth Avenue,
Denver, Colorado 80220

¹ Hoffman, J. R., and McCoy, M. T., *Nature*, **249**, 559 (1974).

² Singer, C. E., Smith, G. R., Cortese, R., and Ames, B. N., *Nature new Biol.*, **238**, 72 (1972).

³ Hoffman, J. L., *Fedn Proc.*, **31**, Abstr. 3690 (1972).

⁴ Frazier, J. N., and Yang, W. K., *Arch. Biochem. Biophys.*, **153**, 610 (1972).

⁵ Jacobson, K. B., *Nature new Biol.*, **231**, 17 (1971).

DR HOFFMAN REPLIES: I concur with Dr Borek when he emphasises that a deficiency in one modified nucleoside in one regulatory tRNA can have profound physiological effects. I contend, however, that if that deficiency is due to altered activity of a tRNA-modifying enzyme, then all tRNAs which are substrates for that enzyme will show the same nucleoside deficiency as the regulatory tRNA. It is then quite likely that nucleoside composition analysis of total tRNA would show a significant deficit in one compartment.

The work of Singer *et al.*¹, which Dr Borek quotes, is an excellent example. It is true that lack of conversion of two Us to ψ s in the tRNA^{His} of the *his T* mutant of *Salmonella* is directly responsible for constitutive derepression of the histidine operon. It is equally true, however, that many other *his T* tRNAs lack this conversion. Singer *et al.*, showed directly that tRNA^{Tyr} and tRNA^{Leu} I and II from *his T* have altered chromatographic mobility when compared with the wild type. They also pointed out that nearly half (5/11) of the *E. coli* tRNA sequences known at that time contained ψ in the same position as in

wild type *Salmonella* tRNA^{His}, presumably as products of a *his T*-like enzyme. If one applies that fraction to Dr Borek's assumed 64 *Salmonella* tRNAs, then in *his T* mutants 32 of them would each be missing two ψ s for a total of 64 (not two) out of 256, a 25% deficit in ψ which would be readily apparent in a comparison of the nucleoside composition of total tRNA from *his T* and wild type *Salmonella*.

Dr Borek also refers to work on the tRNA involved in suppression of the vermilion mutation in *Drosophila*². In a detailed study of the mechanism of this suppression, Twardzik, Grell and Jacobson³ suggest that it is due to lack of an enzyme which modifies tRNA^{Tyr}. They also state that "other tRNAs may be expected to be altered by the hypothetical modifying enzyme". Again I would predict that nucleoside composition analysis of total tRNA from the suppressor strain would show one component significantly deficient in comparison with wild type *Drosophila* tRNA.

Our work on tRNA and ageing has been strongly influenced by Dr Borek's pioneering research on tRNA methylases, in particular the report by Mays, Finch and Borek⁴ on the decline in activity of these enzymes during senescence of rodents. In that report tRNA methylase activity in crude protein fractions, as measured *in vitro* by methyl saturation of total *E. coli* tRNA, declined with age. This decreased activity was attributed to increased glycine N-methyltransferase activity which would produce S-adenosyl-homocysteine, an inhibitor of tRNA methylases. The methods used and the discussion given in this paper were directed toward total tRNA methylase activity and hypomethylation of total tRNA during ageing. Based on this and my above discussion, I still maintain that it was proper for us to search for deficiencies of modified bases in total tRNA as a function of age. Having found no such deficiencies, I must stand on our previous conclusion that tRNA modifying enzymes have no significant role in biological ageing⁵.

Department of Biochemistry,
Health Sciences Center,
Louisville, Kentucky 40201

¹ Singer, C. E., Smith, G. R., Cortese, R., and Ames, B. N., *Nature new Biol.*, **238**, 72 (1972).

² Jacobson, K. B., *Nature new Biol.*, **231**, 17 (1971).

³ Twardzik, D. R., Grell, E. H., and Jacobson, K. B., *J. molec. Biol.*, **57**, 231 (1971).

⁴ Mays, L. L., Borek, E., and Finch, C. E., *Nature*, **243**, 410 (1973).

⁵ Hoffman, J. L., and McCoy, M. T., *Nature*, **249**, 559 (1974).

reviews

SESPA, SCRAM, AIAA *et hoc genus omne*

Scientists and Public Affairs. Edited by Albert H. Teich. Pp. xi+315. (MIT Series in Comparative Politics.) (MIT: Cambridge and London, 1974.) \$14.95.

THIS highly readable volume has, in essence, three themes: scientists *en masse*, scientists as politicians and politicians as scientists. These themes are adumbrated by five authors, eased to the reader's attention by a general preface, five individual forewords by as many pundits, and a postface. It is an intriguing practice: perhaps the run-of-the-mill multi-author scientific text would be more digestible if consumed with an antipasto of comprehensible individual forewords!

The first essay, by Daniel Rich, is concerned with the ethos and functioning of (American) scientific and technical societies. For purposes of exemplification he depends especially on two bodies concerned with meteorology (AMS) and aerospace (AIAA), with glances at physicists, chemists and others. One hotly contested theme is the role of such societies in connection with professional status. Whereas engineering societies, and more especially legal and medical bodies, have a professional licensing function, scientific societies, in America at least, initially fought shy of this but are beginning to follow suit. It is most interesting to see this contentious topic dealt with by a competent political theorist who casts a cold eye on professional pretensions. A few quotations will convey the flavour: "Competition is anathema to professionalism . . ." "The AMS code [of professional ethics] rarely has been enforced". "The public may be protected [by a code] but the profession is also protected by the fiction that all licensed professionals are competent and ethical until found otherwise by their peers".

Another theme discussed by Rich is the relationship between the membership, the elected officers and the permanent officers of a scientific society. He believes that the form of democracy often exists without the substance: the permanent staff are apt to have disproportionate influence. Unlike the Civil Service so bitterly attacked by Marcia Williams, however, it seems that the political 'masters' of the full-time officers are quite content with the arrangement, because generally there is little pressure for change. American

scientific societies are only slowly beginning to become involved with national politics (the third theme of the essays) and there is strong resistance to such involvement. Possibly, when this trend develops, as it certainly will, there will be more conflict between elected and appointed officers.

The second essay is by Anne Hessing Cahn, and concerns the involvement of American scientists in the controversy that raged through Washington a few years ago about the proposed deployment of anti-ballistic missiles. Her study was based upon detailed interviews, or correspondence, with a sample of 152 leading scientific participants in the controversy: identifying the sample seems to have been a research exercise in its own right. The 152 scientists are classified in all sorts of ways—according to disciplines, type of employment, 'distinction' measured in various ways (including the number of citations of their papers), connection with the political establishment, political views, and of course their attitudes to the ABM, and all sorts of cross-correlations are established. The interest of this chapter lies at least as much in the individual vignettes and insights presented in vigorous prose, as in the general conclusions drawn, and it well deserves reading. The 'insiders', including several members of PSAC, the President's Scientific Advisory Committee, were deeply indignant at the way that their advice was either not requested or else ignored, and it seems that though formal advice to the president is protected by secrecy, several advisers came to think that they were entitled to publicise their personal views. The most active campaigners, however, were 'outsiders'—that is, people neither working in the missiles industry nor yet in the inner advisory circles in Washington—opposed to the ABM; some of them were hostile to 'insiders' as a class (*vide* Professor Dash's bold assertion: "Inside advisors are prostitutes"). The outsiders' influence seems to have been, not on the executive, but on the general public, whom they energised to form active groups like SCRAM (Sentinel Cities Reject Anti-Missiles) and on members of congress. One is driven to wonder whether British activists could reasonably hope for a comparable effect by convincing members of parliament.

David Nichols next presents an essay on the various bodies which were set up specifically for scientists, either in isolation or in cooperation with non-scientists, to exert political pressure. This essay returns to the theme touched on in the first chapter, the debate within the conventional societies as to the appropriateness of political action under their auspices. (It seems, from both chapters, that physicists are still in the van of political activists.) Up to now, most political action seems to be the preserve of specially constituted bodies such as the Federation of American Scientists (FAS), or Scientists for Social and Political Action (SESPA). These range from properly organised and financed lobbying bodies such as FAS to bodies whose principles will sound very familiar to academics nowadays; SESPA leaders assert that their group is a "non-organisation—a group with no officers and no constraints on membership", for "if groups are to struggle against non-participatory, undemocratic structures, it is necessary that they don't replicate such structures in their own organising". Such bodies often prefer concentrated local action. Nichols, who is sceptical of the efficacy of such groups in influencing the executive branch (he does not discuss the legislature) remarks: "How local action, not to mention local action by a non-organisation, can combat a ruling elite is, to say the least, not self-evident". There are lessons to be learnt by comparing Nichols's evidence and conclusions with those offered by Anne Cahn in the preceding chapter.

Albert Teich next offers a fascinating survey of the attitudes and modes of life of the multi-national scientific staff of a number of European laboratories, foremost among them the ESTEC (space) laboratory in the Netherlands, CERN near Geneva, and Ispra, a former Euratom laboratory by Lago Maggiore in Italy. They range from the high morale and well-defined function of CERN to the disorganisation and uncertainty of Ispra, both of which emerge clearly from many interview statements. This chapter does not lend itself to summary, but makes most instructive reading, especially for those whose attitudes to European integration have congealed. The one generalisation which can safely be made is that national scientists readily acquire a European outlook in these laboratories (even the indifferent ones); even most

of the British, poor linguists that they still are, become more European in outlook when exposed to high concentrations of 'foreign' colleagues.

The last chapter, by the late R. David Gillespie, is devoted to the politics of cybernetics in the Soviet Union. Norbert Wiener's pioneering ideas of the forties first ran foul of the Great Scientist's henchman, Zhdanov, on grounds based partly on the orthodoxies of dialectical materialism, and partly on the not-invented-here syndrome. Later, cybernetics became part of Soviet orthodoxy. In the words of David Lerner's introduction, "Gillespie charts the shift of official policy from dogged antagonism to euphoric embrace". This is a piece of very solid detailed politico-scientific history.

In the postface (this must be one of the few nearly Latin words invented in the twentieth century!), Eugene Skolnikoff outlines the increasing involvement, since the Second World War, of MIT in the field of science and public policy.

Overall, these essays, which although properly quantitative in approach are refreshingly clear and free of sociological jargon, go down as easily as a dish of oysters. In consuming them, however, one should carefully extract the pearls of wisdom which one will certainly encounter in the process.

ROBERT CAHN

Statistics in ferment

Theory of Probability: A Critical Introductory Treatment. Vol. 1. By Bruno de Finetti. Translated by Antonio Machi and Adrian Smith. Pp. xix + 300. (Wiley Series in Probability and Mathematical Statistics.) (Wiley: London and New York, April 1974.) £7.50.

THE foundations of statistics are in dispute, and have been for many years. Scientists for the most part think they need—and believe they can have—an objective language of statistics by which they can evaluate and communicate their results. Usually they use significance tests, estimation procedures, and confidence intervals, and are happy to overlook the fact that most enquirers into the logical basis of this so-called 'repeated-sampling' methodology have concluded that it is unsound. R. A. Fisher, geneticist as well as statistician, H. Jeffreys, geophysicist, and I. Hacking, philosopher, have been three outstanding critics. Fisher, on whose early work much of the repeated-sampling development relies, was fulsome in his condemnation of later developments, and in *Statistical Methods and Scientific Inference* (1956) sought to stem the tide, allowing, apart from the simple test of significance, only those repeated-

sampling methods which he thought made full use of all the information in the sample. Not only was he unable to provide a unitary method for statistical inference: he formed the opinion that no such method was possible. Jeffreys, who had never regarded the repeated-sampling theory as more than a passing aberration, developed in *Theory of Probability* (1939), an account of the objective Bayesian theory, in which the probabilities of hypotheses are modified by the likelihood obtained from experimental results, according to Bayes' Theorem. Hacking, surveying the whole field in 1965 in *Logic of Statistical Inference*, opined that there was much sense in what both Fisher and Jeffreys had to say, especially in criticism of the repeated-sampling theory, and concluded that the one element common to both their philosophies—the likelihood—could be elevated into a method in its own right, and, furthermore, that it could be extended a little into the repeated-sampling domain to give a satisfactory explication of the core of Fisher's fiducial theory and Jeffreys's theory for the estimation of location and scale parameters.

As if this was not controversy enough, there are those who say that an objective theory of scientific data analysis and reporting is impossible (and who can blame them for pointing to the existing confusion as evidence?). Though the point of view has its origins in the writings of J. M. Keynes and F. P. Ramsey, its principal exponents have been L. J. Savage (*The Foundations of Statistics*, 1954) and Professor Bruno de Finetti. In the present volume the latter offers the first half of his "necessary document for clarifying one point of view in its entirety"—the subjective Bayesian point of view. The work was first published in Italian in 1970, and for English readers has been divided into two (as well as being most admirably translated).

Nobody should attempt to judge this work without prolonged reflection. It must be bought, read, and then left lying around so that it can be assimilated over the years. No book on statistical inference and the foundations of probability ever persuaded anybody directly, for we all make up our own minds by subjecting them to a thousand subtle influences. Only once we are persuaded do we see the virtues of a clear and logical development; Professor D. V. Lindley may be cited as an example, for he writes, in a foreword to this volume, 'that it is a book destined ultimately to be recognised as one of the great books of the world'. A reviewer, mindful of Pascal's wager, might be well advised to agree; but I have my doubts. De Finetti's volume 1 presents only half his case, and since his discussion of inductive reasoning

and statistical inference is not reached until towards the end of volume 2, we must bide our time. What is already certain, however, is that de Finetti is giving a masterly clarification of 'one point of view in its entirety', raising the level of discussion to unsurpassed heights. I await volume 2 with impatience.

A. W. F. EDWARDS

Population structure

Methods and Theories of Anthropological Genetics. Edited by M. H. Crawford and P. L. Workman. Pp. xviii + 509. (A School of American Research Book.) (University of New Mexico: Albuquerque, December 1973.) \$20.

WHAT can 'anthropological genetics' be? That part of genetics pertaining to man, usually called human genetics? No, this book is really about genetical anthropology, or that part of anthropology whose study is made more fruitful by an appeal to genetical principles and genetical information, a sibling of social anthropology and physical anthropology. In particular it is the study of population structure and population relatedness using models and methods developed by geneticists, and gene frequencies as a primary source of information. It arose because of the conjunction of a vast increase in the amount of genetic information available and the advent of electronic computing, and has particular topicality because of the modern discussion of racial differences.

About ten years ago some geneticists with a mathematical bent started applying their techniques to some of the problems of anthropology, and began developing new techniques appropriate to the computer age. Although anthropologists had earlier assimilated the pioneering work on the distribution of the human blood groups, they tended to regard this incursion with suspicion, but soon came to terms with the new approach. The success of the invaders owed much to the fact that not only did the geneticists bring their own tools: they also brought a wide range of statistical and computational expertise that was, for the most part, new to anthropology.

This volume is a revised and extended report of a conference held in early 1971, and consists of nineteen papers (the last being a useful review of the remainder) followed by nearly six hundred references. These are interesting in themselves: a histogram for the years of publication shows a close fit to an exponential rate of increase with a doubling time of five years (20 in 1960, 40 in 1965, 80 in 1970), and is an indication of the impossibility of being really up to date with such a book.

The volume is an excellent summary of the present state of play in the field, and would provide a good entry for anyone wishing to join in. There are one or two notable absentees from among the contributors, but readers will soon be led to their work through the references. It is difficult to escape the impression, however, that genetical anthropology is at a somewhat adolescent stage, reflected by methodological controversies about matters of no great moment, and by the fact that in all that has been written one can discern nothing that is really surprising, no great contribution to knowledge or understanding that takes the subject beyond the merely documentary. It is possible, indeed probable, that once the field has settled down something will be forthcoming, and this book may serve as a useful appetiser.

A. W. F. EDWARDS

Pharmacology of neurones

Transmitters and Identified Neurones in the Mammalian Central Nervous System. By Andris K. Tebécis. Pp. xvi + 340. (Sciencetechnica: Bristol, May 1974.) £11.50.

MANY books and symposia have appeared in recent years on the subject of neurotransmitters in the mammalian central nervous system, but Dr Tebécis's monograph deals with the topic in a highly original manner.

One of the most powerful techniques for studying the actions of transmitter substances on neuronal receptor sites in the brain or spinal cord involves the recording of the electrical activity of single neurones while applying transmitters and other drugs in a highly restricted manner on to the surface of the cell from which such recording is being made. Such micro-applications are achieved by the electrophoretic ejection of minute quantities of chemical from the fine tip of a glass micro-electrode situated near the cell surface. The effects of several different substances may be tested on the electrical activity of a single neurone by using multibarrelled arrays of such micro-electrodes. Such studies allow one to define the neuropharmacological properties of individual neurones in the CNS; that is, whether they are capable of responding to various possible transmitters, and if so to define what particular type of transmitter receptor is involved. This approach can be used to determine which transmitter when applied to a neurone most closely mimics the response seen when transmitter release is induced naturally by stimulating one or other of the neurone's presynaptic connections. In this way one may determine the

identity of the transmitters used by various neuronal pathways in CNS. These experimental approaches have been widely used in recent years, and a bewildering plethora of results is available in the literature.

Dr Tebécis has undertaken the Herculean task of collating much of this information into a meaningful pattern. He has been helped in this by the novel approach he has taken. It has become increasingly clear that the neuropharmacological properties of different types of neurone in the CNS can vary widely. Results obtained by the micro-iontophoretic application of drugs and transmitters to randomly selected single neurones in any given region of the brain are thus likely to be extremely difficult to interpret, since several different neurone types are likely to be recorded from. Far more consistent results are obtainable, however, if recordings are made selectively from only one type of neurone in any given brain region. This may be done, for example, by using only neurones which can be identified as responding to antidromic stimulation of their axonal bundles at some remote site in the nervous system. For example, spinal cord motor neurones may be identified by their antidromic response to the electrical stimulation of motor nerves.

Dr Tebécis reviews what is known from neurophysiological studies of the pharmacological properties of "identified" neurones in various areas of the CNS. Successive chapters deal with the spinal cord, brainstem, cerebellum, diencephalon, basal ganglia, limbic system, cerebral cortex and retina. In each chapter detailed discussion is given of the results available for each of the various identifiable neurone types from which single unit recordings can be obtained. In the brain stem, for example, fifteen different categories of neurone are described. In each case any biochemical or histochemical data that are available concerning the distribution of transmitter-specific neuronal pathways are also reviewed. The emphasis is on the actions of acetylcholine, the excitatory amino acids, GABA, glycine, catecholamines and 5-HT. The author, however, also indicates the large gaps in our knowledge of the pharmacology of cerebral circuitry, pointing out the many instances in which the identity of the transmitters used at CNS relays remains unknown. The book is well illustrated, and very up to date. The bibliography is extensive and will be a valuable collection of source material for those interested or involved in this field.

The organisation of material in the book means that it has more the nature of a catalogue of useful information than a monograph, but the catalogue

will nevertheless prove very useful to many neuroscientists. The author assumes that the reader has a fairly high level of knowledge of the physiological and pharmacological workings of the nervous system, so the book will appeal mainly to a small number of scientists or graduate students with this necessary expertise and interest. They will, however, be grateful to Dr Tebécis for his critical and authoritative review.

LESLIE IVERSEN

Science lessons

Science for the People: The Origins of the School Science Curriculum in England. By David Layton. Pp. 226. (Allen and Unwin; London, January 1974.) £3.55.

PROFESSOR LAYTON'S well-written book ought to be made compulsory reading for all those scientists, administrators, civil servants and politicians who have anything to do with science education.

By looking at the Victorian roots of the "Science for the people" idea, Layton has exposed many of the fundamental tensions in science curricula which still plague us. From the beginning there were clashes in attitudes towards both method and ideology of science teaching. What was science education for? Should it teach the "science of common things", or be more concerned with "applied" goals? Each viewpoint was intimately connected with political and social ideas. Through studies of Richard Dawes, J. S. Henslow, Henry Moseley and other educationalists, and through curriculum analysis, plus some good political history, Layton has been able to synthesise a view of the past which has direct relevance today.

There are, however, several things in the book which indicate a slight historical amateurishness. Anachronistic social theory is one. The book claims, for example, that amongst some educationalists of the 1850s, "Elementary schools were conceived primarily as instruments of social control". Possibly true, but this is much too theory-laden for the historian's palate. The general aim of the book is unhistorical, too. Layton has written the first seven "historical" chapters largely as a background to an almost totally theoretical chapter 8.

But this is no more than a professional quibble. The book itself has a value far greater than that of most historical works. And Professor Layton has done science education an extremely important service.

JAMES FRIDAY

Protozoa with lashes

The Ciliates. By A. R. Jones. Pp. 207. (Hutchinson: London, April 1974.) £3.50 boards; £2 paper.

My first impressions of this little book were similar to those of a research student who, in the local bookshop, announced that it looked dull and uninteresting. But first impressions can be misleading and I subsequently discovered the written content to be of a better quality than the visual appeal.

The subject matter of the book is wide including chapters on the form, fine structure, locomotion, feeding, nutrition, reproduction, growth, taxonomy, and economic importance of ciliated protozoa. In general these chapters are readable, interesting and up to date although in places the style is rather slack. A complete chapter devoted to osmoregulation and ion control is a refreshing feature not usually included in such textbooks and reflects the personal interests of Dr Jones. The book is obviously written with some enthusiasm for the subject and I found several pieces of new and useful information not included in other texts. I am not enthusiastic about the general format of this series of books and in this one I would have liked to have seen more original illustrations. In several places I felt that a good diagram would have made the description simpler and more concise.

As in all modern textbooks there is the inevitable series of errors scattered throughout the text. Some although minor could be misleading, for example, referring to the Ciliophora as a subphylum and a class on the same page, the invention of a new genus *Campanula* (for *Campanella*) and some erroneous references. Although I remain unclear at whom the book is really aimed I can recommend it to all those interested in ciliates since it does make a useful addition to the small but rapidly growing set of books dedicated to protozoological topics.

COLIN R. CURDS

Dying of cold

Temperature and Life. By H. Precht, J. Christophersen, H. Hensel and W. Larcher. Pp. xx+779. (Springer-Verlag: Berlin and New York, 1974.) DM 142; \$58.30.

Effects of Temperature on Ectothermic Organisms: Ecological Implications and Mechanisms of Compensation. Edited by Wolfgang Weiser. Pp. xi+298. (Springer-Verlag: Berlin and New York, 1974.) DM 66; \$23.10.

TEMPERATURE is one of the most important and easily measured environmental influences affecting living organisms. Many aspects of physiology

are best understood in terms of reactions to stress and, of the various factors causing stress, temperature is, perhaps, the one most often encountered. Study of thermoregulation has contributed much to the concept of control theory in biology: by the same token, investigation of thermal adjustment in ectothermic animals is likely to have far-reaching influence on ideas about the regulation of metabolism in general. For reasons such as these, various aspects of thermobiology are today being studied extensively, with the result that new books and review articles are constantly appearing. The volumes under review are the latest on the subject to appear.

The first is a revised and updated English version of *Temperatur und Leben* by Precht, Christophersen and Hensel, first published in 1955. Although constructed along the same lines as its predecessor, it differs in that the number of contributors has been increased to include two microbiologists, one biochemist, seven botanists, three zoophysicologists and three human physiologists. An attempt is made to treat as many problems as possible, but the main theme remains the adaptation of organisms to changing temperatures. A supplementary volume on physical and chemical aspects by L. Lumper, is to be published later. A special effort has avowedly been made to cover, often in the form of footnotes, the copious literature that has appeared since 1955. To judge by those particular fields with which I am familiar, however, it would seem unlikely that more than about one tenth, at most, of significant contributions has been included. Even so, a rather turgid compendium has been produced, probably of use to research workers for reference purposes rather than for stimulation, whose detail at the same time far exceeds the requirements of most graduate students.

The various chapters have been grouped under the headings of poikilothermic organisms (microorganisms, plants, animals) and homeothermic organisms. In my view there is no clear distinction between homeothermy and poikilothermy, so the classification adopted tends to obscure the relationship between homeothermy and size in animals. With an unfavourable surface to volume ratio, the maintenance of a constant body temperature would be economically unacceptable for small organisms.

Temperature and Life shows little evidence of the meticulous planning that clearly went into its nearest competitors such as: *Temperature Regulation in Mammals and other Vertebrates* by J. Bligh, (Elsevier, North-Holland: Amsterdam, 1973); *Cryobiology* edited by H. T. Merryman (Academic Press:

London and New York, 1966); *Thermobiology* edited by A. H. Rose (Academic Press: London and New York, 1967); and *Comparative Physiology of Thermoregulation*, vols 1–3, edited by G. C. Whittow (Academic Press: New York and London, 1970, 71, 73). Of course, none of these covers quite such a wide field as the new book, although their design is far better. Indeed, herein lies the nub of my criticism. The study of temperature and life has long since passed the point at which it can be reviewed comprehensively in a single volume—even one of 779 pages with fine type.

Wolfgang Wieser's book likewise suffers from the fact that it possesses little intrinsic homogeneity. This, however, is probably unavoidable, since it represents the proceedings of a symposium held at Obergurgl, Austria, from September 4–8, 1972, at which only a restricted number of topics was covered. The 25 contributions are grouped under three headings: mechanisms, ecology and cold resistance but, within these broad classifications there is often little in common between one paper and the next. There is also very considerable variation in quality. An opening review by the editor stresses the complex "multistable system" from which the answer best suited to the prevailing or anticipated thermal régime is called into action by changes in ambient temperature. This farsighted approach may well provide the kind of stimulus lacking from *Temperature and Life*.

Despite the vast amount of data reviewed in both books, it is evident that knowledge of thermobiology is still superficial. To cite a few examples, the mechanisms by which death results from heat or cold are very imperfectly understood. They are certainly diverse and are complicated by the fact that unrelated mechanisms within an organism may be influenced concurrently in different ways. Extremes of temperature may denature enzymes, or liquify membrane lipids. The injurious action of heat may also damage mitochondria, affect the coding of proteins, impair the coordination of physiological processes, disturb the functioning of endocrine glands and neurosecretion, and engender the accumulation of harmful metabolic products or an inadequacy in the transport and supply of oxygen. The harmful effects of cold are equally multiple. Again, little is known of the neurophysiology or mode of function of thermal receptors in animals, nor why long-term adaptation has so little effect on the properties of these sense organs. It would be misleading to lump together a number of unrelated phenomena merely because they are engendered by the same thermal influence: one must therefore accept the fact that no unitary explanation exists.

The indexes of both volumes are restricted to subject only: the titles are omitted from references cited in the first. Since these number well over 4,000 this defect is, no doubt, unavoidable.

J. L. CLOUDSLEY-THOMPSON

Systematic archaeology

Kalambo Falls Prehistoric Site. Vol. 2: The Later Prehistoric Cultures. By Desmond J. Clark. With contributions by B. M. Fagan, M. R. Kleindienst and F. van Noten. Pp. xiv+420. (Cambridge University: London, February 1974) £17; \$49.50.

THIS is the second volume of Professor Clark's report on the archaeological sites at Kalambo Falls on the borders of Zambia and Tanzania. The first covered geology, palaeoecology and stratigraphy (reviewed *Nature*, 223, 326, 1969); the second covers the upper part of the archaeological sequence, from the Iron Age back to the Second Intermediate, about 7600 BC. The third volume, still in preparation, will cover the earlier periods, ranging in date from about 25000 BC to more than 60000 BP. Both published volumes reflect the modern standards required for excavation reports. Very careful excavation of a number of sites has produced a remarkable range of information particularly in the environmental field; further, a number of carbon-14 dates have been obtained covering a long period, dates which are much needed in African prehistory.

The upper part of the archaeological sequence, the Iron Age, covers a time span from about AD 345 to AD 1580, the last date apparently being that of the foundation of the modern settlement in the district. This continuity of occupation is discussed in chapter 1, where the history of the recent Bantu settlers is dealt with in some detail. Chapters 2 and 3 cover the Iron Age settlements and chapters 4 to 7 inclusive the two Stone Age complexes which precede them.

The latest of these Stone Age industries has carbon-14 dates of around 1950 BC and belongs to the "Late Stone Age" of southern Africa. Like many complexes grouped under this heading the Kaposwa, as Clark calls this material, is characterised by an abundance of microlithic tools, mostly crescents and triangles. This Kaposwa has been compared with a number of sites in Zambia, Malawi and further south which are within the same time range and which have a comparable industry; some of these seem to be as early as 10000 BC, similar in age to the early postglacial industries in Europe.

The trend towards microlithic tools continued in parts of South Africa into early colonial times among groups which had retained a strictly hunting and food-gathering economy.

Clark named the other stone age industry Polungu. It belongs to a phase which in southern Africa is referred to as the Second Intermediate; a stage between the Middle Stone Age with industries based on a flake technology and the frequent use of prepared cores, and the Late Stone Age with microliths and a blade technology like the Kaposwa. The Polungu from Kalambo Falls comes from two stratigraphic units. So far only the earlier has produced a carbon-14 date, about 7600 BC—towards the end of the time range for these industries which probably go back to about 1600 BC. This period was within the Late Glacial in Europe.

Both Kaposwa and Polungu differ in some respects from other Second Intermediate industries, not surprisingly considering the time and distance separating them. The Second Intermediate industries do, however, make a fairly homogeneous group.

Clark treats all three complexes in great detail with a minute statistical breakdown and a discussion of each in relation to its place in the African sequence, thus providing an informative summary of later African prehistory. He has also given a great deal of space to his own views on classification so his terminology is clear. Every ounce of information possible has been extracted from the excavation and presented in a very systematic manner with an excellent standard of book production. I have only very trivial complaints: plate 98 seems of little value and the caption of plate 97 could have been a little clearer.

J. WAECHTER

Biology called to account

The Scientific Conscience: Reflections on the Modern Biologist and Humanism. By Catherine Roberts. Pp. xii+130. (Centaur: Fontwell, Sussex; July 1974.) £2.50.

MANY people have uncomfortable feelings of moral ambivalence when they consider the aims, practices and ideas of much current science, and particularly of the self-styled "new biology". The leading figures in this domain may be admired or envied for their energy, cleverness, and social success, but few would look to them as exemplars of the primary human virtues. The modesty of a Darwin, the personal courage of a J. B. S. Haldane, the altruism of an A. R. Wallace, the sense of public

responsibility of a Pasteur, or the dedicated persistence in the face of poverty or ill health or both, displayed by many humble naturalists of the last century, are all qualities hard to match among the biological celebrities of our day. It is scarcely accidental that several recently published books on the philosophy of biology make no reference to ethics at all, but treat science as an entirely autonomous domain. The moving spirit of modern biology has a decided resemblance to that Mephistopheles who in the prologue to Goethe's "Faust" presented himself before God in true Popperian fashion as "the spirit that denies", and later in the play was constantly urging Faust to "the effecting of all things possible" by demonic aid.

Dr Catherine Roberts, a microbiologist turned to classical Greek studies and philosophy, has gathered together a number of essays, the chief unifying factor among which is that they are all directed against various aspects of the Mephistophelean spirit in modern biology. The latest of the essays dates from 1966, so that we do not have Dr Roberts's reactions to *The Double Helix* of J. D. Watson, which might have been interesting. Her own standpoint, nowhere clearly stated in this book, would seem to be a form of neo-Platonism like that of Kathleen Raine, and very definitely committed to the cause of God as against Mephistopheles. Her basic attack is directed against the whole concept of science as a completely autonomous activity. Despite the title, her argument implies that there cannot really be such a thing as a 'scientific' conscience; if we make moral judgments and valuations, we cannot do this *quâ* scientists, but only in what she considers to be our higher capacity as fully human beings.

The essays are essentially separate, and inevitably somewhat overlapping; they express, at times with some eloquence, a consistent point of view, and one which is likely to gain more adherents in future, but do not attempt a systematic intellectual exposition of it—for this, with engaging modesty, Dr Roberts refers her readers to Michael Polanyi's well known *Personal Knowledge* (1958). The whole book has what used to be thought of as a 'feminine' quality, basing itself more on an appeal to the heart than on coercive rationalisation. Her opponents will doubtless deny the validity of any such appeal, and take credit for their own heartlessness. We may well wonder for how long it will be possible for such people to pursue ambitious scientific careers, financed at the expense of taxpayers, half of them women, and most of them with old fashioned human hearts.

R. A. CROWSON

Microbes and men

Microbes and Men. Producer: Peter Goodchild. BBC2, Wednesday 9.55 pm.

FOR their newest medical/scientific effort, BBC television have homed in on four of the founding fathers of modern medicine and bacteriology, and by means of carefully reconstructed dramatised documentary have tried to tell it as it was. Unfortunately, in the process they have failed to enliven these undoubtedly fascinating characters; Semmelweis, Pasteur, Koch and Ehrlich.

Locations, medical instruments, crucial experiments and the appalling conditions in hospitals at that time are painstakingly reconstructed, and the commentary linking the episodes of dramatic action is lucid, informative and accurate. They have foregone the image of the great scientist and have faithfully documented the petty squabbles, the failures and the obtusenesses

as well as the brilliant insights and triumphs.

The fact that in spite of all this the programmes never come alive may be a fault inherent in the presentation itself. Patches of dramatic dialogue are interspersed with the commentary, which although necessary to convey all the hard facts and point the moral in the 55 minutes allowed for each episode, throws an air of unreality over the whole proceedings, especially as the marriage of commentary to picture is sometimes heavy-handed.

The first programme of the series, on the Hungarian doctor Ignatz Semmelweis, screened this week, may, in fact, be the best. Semmelweis was an exceedingly complex character, especially in his failure to follow up his brilliant work on the origins and transmission of puerperal fever, a disease which killed one in three women in childbirth in the 19th century lying-in hospitals. As one character says to Semmelweis'

wife in the 'authentic' context of the cosy drawing room, "Science is moving, and I want to make sure he doesn't get left behind". The programmes are in fact littered with this sort of aphorism, usually delivered in ringing tones. The overall effect, for all its undoubted authenticity, is remarkably reminiscent of the great 'lives of the composers' genre of films.

The BBC have seen fit to screen the series relatively late in the evening although the programmes would be eminently suitable for children and would probably be much enjoyed by them. Perhaps the BBC thought that the blood and gore, especially in the first episode might be off-putting, but children are usually far more resistant than the adult audience for whom it is obviously intended.

A book of the series (*Microbes and Men*, £2.50) will be available next month.

ELEANOR LAWRENCE

obituary

H. Taylor

SIR HUGH TAYLOR, former graduate school dean and chairman of the chemistry department at Princeton University for 25 years, died at Princeton on April 17, 1974 at the age of 84.

Born in St. Helens, Lancashire, he received his University training at Liverpool under the tutelage of Sir Frederick Donnan, graduating in 1909 and earning a masters degree in 1910. In 1912 and 1913 he studied reaction kinetics, the first year at the Nobel Institute at Stockholm with Svante Arrhenius and the second with Max Bodenstein at Hanover Technische Hochschule. In 1914 he was awarded a doctorate at the University of Liverpool. He went from there to Princeton University, where he spent the rest of his life except for two years during the First World War when he worked in the United Kingdom.

In Princeton, Taylor progressed

rapidly through the early professional grades becoming a professor in 1922 and Chairman of the Department of Chemistry by the time he was 36. He served in this capacity from 1926 to 1951. He also served with distinction as Dean of the Graduate School from 1945 to 1958. A person as effective as Sir Hugh has no opportunity to retire. After 1958 he continued his dedicated interest in graduate education serving as the first president of the Woodrow Wilson National Fellowship Foundation, the Princeton-based foundation supporting quality in higher education.

Sir Hugh's influence on kinetics has been felt worldwide, especially as applied to catalysis which is a theme running through his 300 scientific papers and his books. The book he wrote with E. K. Rideal *Catalysis in Theory and Practice* published in 1919 grew out of work by the authors in

Britain during the First World War and was widely used. His contributions to photochemistry, radiochemistry and chemical kinetics were likewise fundamental. During the Second World War, his pioneering efforts in preparing heavy water and diffusion barriers were of great importance to the atomic bomb project.

No picture of Sir Hugh would be complete which left out his generosity, his intense vigour and his decisiveness. He usually emerged from his office just slightly slower than running. In the seminars which he presided over so superbly, no one could present more quickly and clearly the gist of the problem at hand and place it in proper perspective. With this went a warm enthusiasm and an appreciation of good work by others that made reaction kinetics, and chemistry in general, blossom profitably at Princeton.

Announcements

Erratum

In the article "Activation of suppressor T cells by tumour cells and specific antibody" by R. K. Gershon *et al.* (*Nature*, 250, 594; 1974) the end of one sentence and the beginning of the next were inadvertently omitted from the penultimate paragraph. The third and fourth sentences of that paragraph should read 'It is well known that the

immunogenicity of antigen is highly dependent on the physical state in which it is administered¹⁹. Antibody can affect the physical stage of antigen²⁰ and in so doing, perhaps, change the T cell response to it.'

Reports and Publications

Great Britain

The Zoological Record, 1970, Vol. 107, Section 6: Vermes. Part C: Conodonts. Fossil Vermes. Trace Fossils. Compiled by S. Ware. Pp. 22. £3.65. 1970. Vol. 107, Section 13: Insecta. Compiled by the Staff of the Zoological Society of London. Pp. x + 729. £12.15. (London: The Zoological Society of London, 1974.) [107]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 267, No. 890: Biomechanics of *Pteranodon*. By Cherrie D. Bramwell and G. R. Whitfield. Pterodactyls Past and Present. By D. M. S. Watson. D. M. S. Watson's Notes on Pterosaurs. By Cherrie D. Bramwell and G. R. Whitfield. Pp. 503-592 + plates 24 and 25. (London: The Royal Society, 1974.) [117]

Other Countries

Lawrence Livermore Laboratory in Brief. Pp. 24. (Livermore, California: Lawrence Livermore Laboratory, 1974.) [87]
Smithsonian Contributions to Zoology. No. 139: Gammaridean Amphipoda of Australia, Part. II By J. Laurens Banard. Pp. v + 148. \$2.20 No. 159: Clind Fishes of Chile and Peru, with Description of a New Species, *Myxodes ornatus*, from Chile. By John S. Stephens, jun and Victor G. Springer. Pp. 24. 70 cents. (Washington, DC: Smithsonian Institution Press, 1973 and 1974. For sale by US Government Printing Office.) [87]